MATTEO TRIMARCHI

Curriculum vitae

2022

RIASSUNTO DEL CURRICULUM

Il Prof. Matteo Trimarchi ricopre l'incarico di Professore Ordinario di Otorinolaringoiatria alla Facoltà di Scienze Biomediche dell'Università della Svizzera Italiana (USI) e Primario di Otorinolaringoiatria dell'Ente Ospedaliero Cantonale Lugano.
Precedentemente professore Associato della Facoltà di Medicina e chirurgia in otorinolaringoiatria (dal 2015), direttore della Scuola di Specialita' in Otorinolaringoiatria dell'Università-Vita Salute di Milano (dal 2022) e dirigente medico senior di otorinolaringoiatra dell'Unità di Otorinolaringoiatria dell'IRCCS Ospedale San Raffaele a Milano (dal 2002).
Dopo la laurea in Medicina e chirurgia presso l'Università di Pavia nel 1996 con 110/110 con lode, si specializza in Otorinolaringoiatria presso l'Università di Brescia nel 2001 con 50/50 con lode.
Ha ottenuto l'abilitazione a professore di prima fascia il 5.12.2017.
Per quanto concerne l'approfondimento professionale, il professor Trimarchi ha svolto periodi di frequenza e studio presso centri Ospedalieri e Universitari (fra i quali si ricordano il S. Thomas Hospital and Guy's Hospital di Londra, il Department of Anatomy, Harvard University, di Boston, il Department of Otolaryngology, Mayo Clinic di Rochester e il Royal National Throat Nose and Ear Hospital, University College di Londra).

Dal 2002 ad oggi ha effettuato più di 7000 interventi nell'ambito della patologia testa e collo. Ha svolto ricerche in vari settori della specialità, orientate in particolar modo allo studio della patologia e della chirurgia endoscopica naso-paranasale e allo studio dei tumori della testa e del collo. Di particolare interesse i risultati ottenuti nello studio delle lesioni distruttive centro-facciali da abuso di cocaina, delle patologie naso sinusali e dei disturbi delle vie lacrimali.

È autore di più di 100 pubblicazioni su riviste nazionali ed internazionali, di un manuale di otorinolaringoiatria, di 5 capitoli di libri e di due sistemi di archiviazione dati e immagini dei pazienti con neoplasie dei seni paranasali.

È membro della Società di Otorinolaringoiatria italiana, della Società di Rinologia europea, dell'Accademia di Rinologia italiana (socio fondatore), della Società di Otorinolaringoiatria Spagnola (socio onorario).

- E' stato membro del Board del Gruppo Lombardo di Otorinolaringoiatria (2017-2019).
- E' stato vicepresidente del Gruppo Lombardo di Otorinolaringoiatria (2019-2021).
- E' stato consigliere AUORL nel consiglio direttivo della Società Italiana di Otorinolaringoiatria e Chirurgia Cervico Facciale (2018-2021)

INDICE

1. COMPENDIO DEI TITOLI DI STUDIO E DI CARRIERA

Data di nascita: 25 maggio, 1971

Posto di nascita: Pavia

Indirizzo casa: via Residenza spiga 261. 20090, Segrate, Milan, Italy

Indirizzo lavoro: Unità Operativa di Otorinolaringoiatria, Università Vita Salute, San Raffaele IstitutScientifico, via Olgettina 60, 20132 Milan, Italy

Numero di telefono: ++39 02 26433522

Numero di FAX ++39 02 875644

Numero di cellulare: ++39 347 3548596

Formazione scolastica: Laureato all'Università di Pavia, Italia (1996)
Specializzato in Otorinolaringoiatria all'Università di Brescia (2001)

Posizione accademica e di insegnmento attuale Professore Associato di Otorinolaringoiatria, Università Vita Salute Scuola di Medicina e Chirurgia, San Raffaele Istituto Scientifico, via Olgettina 60, 20132 Milano, Italia

Membro delle seguenti

Società: Società Italiana di Otorinolaringoiatria

Società americana di Otorinolaringoiatria

Società Europea di Rinologia

Società Spagnola di Otorinolaringoiatria

E' stato membro del Board del Gruppo Lombardo di Otorinolaringoiatria (2017-2019).

E' stato vicepresidente del Gruppo Lombardo di Otorinolaringoiatria (2019-2021).

E' stato consigliere AUORL nel consiglio direttivo della Società Italiana di Otorinolaringoiatria e Chirurgia Cervico Facciale (2018-2021)

Compendio dei titoli di studio e di carriera

1990 Diploma di Maturità Scientifica presso il Liceo- scientifico Taramelli Pavia .

1995 Borsa di studio Erasmus della durata di 5 mesi al St. Thomas Hospital di Londra, (5 mesi)

1996 Laurea in Medicina e Chirurgia (110/110 e Lode), Università di Pavia. (Tesi: "Nistagmo Congenito caratteristiche cliniche ed elettronistagmografiche").

1996 Iscrizione Scuola di Specializzazione in Otorinolaringoiatria, Università di Università di Brescia, Italia.

1998-1999 Ufficiale Medico dell'Aeronautica militare, 36° Stormo di Gioia del Colle e 50° Stormo di Piacenza.

2001 Conseguimento del 1° premio per il Video "Endoscopic Dacryocystrhinostomy (DCR) in the pediatric age". 5th international conference on pediatric ORL. Graz 11 luglio

2001 2001 Specialista in Otorinolaringoiatria (50/50 con Lode), Università di Brescia (Tesi "Cocaine-induced midline destructive lesions: clinical, radiologic, histopathologic, serologic exams and differential diagnosis in a group of patients with Wegener's granulomatosis").

2002-2006 Dirigente medico di I Livello, Unità Operativa di Otorinolaringoiatria, San Raffaele Scientific Institute in Milano e tutor in Otorinolaringologia presso Università Vita Salute, San Raffaele di Milano.

2004 Professore a contratto di Otorinolaringoiatria (MED31) (Riinologia) e Statistica medica (MED01) nel programma della scuola di specializzazione in Otorinolaringoiatria (Università Vita Salute, Scientific Institute San Raffaele di Milano).

2007 Dirigente medico Senior, Unità Operativa di Otorinolaringoiatria, San Raffaele Scientific Institute in Milano.

2010 Memnro dell' Advisory Board of the European position paper sulla gestione endoscopica dei tumori del naso dei seni paransali e della base cranica.

2011 Membro Onorario della Società Spagnola di Otorinolarigoiatria

2011-2015 Consulente del dipartimento della politica antidroga della presidenza del Consiglio dei Ministri, Roma, Italia.

Dal 2015 Professore Associato in Otorinolaringoiatria, Università Vita Salute, Ospedale San Raffaele, Milano, Italia.

Dal 2016 Coordinatore the del Corso di Scienze Chirurgiche alla Scuola di Medicina, San Raffaele Università Vita Salute, Milano, Italia.

12.05.2017 Ha ottenuto l'**abilitazione a professore di prima fascia**. (BANDO D.D. 1532/2016, SETTORE CONCORSUALE 06/F3 OTORINOLARINGOIATRIA E AUDIOLOGIA ABILITAZIONE A I FASCIA).

Dal 2019 Presidente della Commissione paritetica Scuola di Odontoiatria, San Raffaele Università Vita Salute, Milano, Italia.

MADRELINGUA: ITALIANA

ALTRE LINGUE: INGLESE

• Capacità di lettura: ECCELLENTE

• Capacità di scrittura: BUONA

• Capacità di espressione orale: ECCELLENTE

CAPACITÀ' E COMPETENZE TECNICHE

(*Con computer, attrezzature specifiche, macchinari, ecc.) :* Sistemi di navigazione medicali, laser diodi, laser Co2, videostrobolaringoscopia, endoscopia
Ambito informatico, Mac, Windows. utilizzo di Microsoft Office, Photoshop, Acrobat professional, Final cut express, iMovie, Keynote, pages

CAPACITÀ E COMPETENZE ARTISTICHE : Flauto traverso, sassofono, pianoforte

ALTRE CAPACITÀ E COMPETENZE : Equitazione, vela, sci neve e acqua, pattinaggio, nuoto, Subacquea.

PATENTE O PATENTI: Patente di guida A e B. Patente di abilitazione al comando di unità da diporto entro 12 miglia

2. SOGGIORNI ALL'ESTERO

1Febbraio –30 Luglio1995

ERASMUS a Londra Department of Neurology Guy's Hospital,
Department of Haematology S. Thomas Hospital
Department of Orthopaedics S. Thomas Hospital
Department of Ophthalmology S. Thomas Hospital
Department of Otolaryngology Guy's Hospital

1 Agosto –1 Settembre, 1996

Department of Anatomy, University of Harvard, Boston, USA

27 Agosto –6 Settembre, 1998

Department of Otolaryngology, Mayo Clinic, Rochester, USA

14–25 Agosto, 2000

Royal National Throat Nose and Ear Hospital, University College of London, England.

6–10 Agosto 2001

Royal National Throat Nose and Ear Hospital, University College of London, England

5-9, Aprile 2004

Royal National Throat Nose and Ear Hospital, University College of London, England

20-22 Aprile, 2009

Royal National Throat Nose and Ear Hospital, University College of London, England

16-17 Febbraio, 2010

Royal National Throat Nose and Ear Hospital, University College of London, England

13-17 Febbraio, 2011 International Biological, Inc., Detroit, Michigan, USA.

3. ATTIVITA' SCIENTIFICA

A) Corsi di perfezionamento

A 01) Corso di Perfezionamento in Rinologia. Pavia 21 Febbraio -18 Ottobre 1997.

A 02) Head and Neck Surgical Pathology Course. Università di Brescia, Brescia, Italia, 9-10 marzo, 1998.

A 03) Master Course on Neck Surgery. Milano, Italy. 29 Novembre - 3 Dicembre, 1999.

A 04) Course on Pediatric Endoscopy. Brescia, Italy. Aprile 18-21, 2000.

A 05) Videocorso di Chirurgia Endoscopica Rinosinusale e Microchirurgia del basicranio "Alessandro Guccione". Pavia, Italy 2-4 Ottobre. 2000.

A 06) XIII Corso Andreas Vesalius. Master Pratico Intensivo di Anatomia Chirurgica Dissettiva e Operazioni – Anatomia Chirurgica e Tecniche Avanzate di Chirurgia Endoscopica Sinusale e della Rinobase – Approcci laterali al Basicranio: Anatomia Chirurgica Operazioni, Tecniche e Tecnologie Emergenti. Bruxelles, 29 Ottobre - 2 Novembre , 2000.

A 07) Corso Residenziale Avanzato. Imaging del cavo orale e dell'orofaringe. Brescia, Italy. 12-15 Novembre. 2000.

A 08) Master Course on Neck Surgery. Milano, Italy. 27-29 Novembre, 2000.

A 09) Chirurgia delle vie lacrimali"da Toti all'endoscopia" Firenze, Italia. 12 Maggio , 2000.

A 10) Penn Rhinology Corse: Advances in Management of Sino-Nasal Disease. Philadelphia, USA. 8-10 Marzo, 2001.

A 11) Master Course on Endoscopic Treatment of Laryngeal Cancer. Milan, Italy. 12.14 Marzo, 2001.

A 12) International Instructional Masterclass. Exploring challenging in Endonasal Endoscopic Microsurgery Rhinoplasty Facial Aesthetic Surgery. Milan, Italy. 25-31 Marzo, 2001.

A 13) Second International Copenhagen Corse on Computer Aided Endoscopic Surgery of the Paranasal Sinuse and Skull Base. Copenhagen, Denmark. 5-6 Aprile, 2001.

A 14) Second Intensive Hands on Dissection Corse on Skull Base Surgery: Surgical Approach to the Sellar Tumors. Milan, Italy. 17-19 Maggio, 2001.

A 15) 28th International Workshop on Functional Endoscopic Sinus Techniques, Graz, Austria. 2001.

A 16) Secondo Videocorso ”Alessandro Guccione” – Roncochirurgia - Chirurgia Endoscopica Rinosinusale e del Basicranio. Varese, Italy 3-5 Ottobre, 2001.

A 17) Corso Pratico Intensivo di Anatomia Chirurgica Dissettiva e Operazioni Moderne Tecniche Fondamentali di Chirurgia Rinologica, Bruxelles Novembre 2001.

A 18) Corso Pratico Intensivo di Anatomia Chirurgica Dissettiva e Operazioni Moderne Tecniche Fondamentali di Chirurgia Rinologica, Bruxelles Novembre 2002.

A 19) Corso Pratico Intensivo di Anatomia Chirurgica Dissettiva e Operazioni Moderne Tecniche Fondamentali di Chirurgia Rinologica, Bruxelles, Novembre 2003.

A 20) Corso Pratico Intensivo di Anatomia Chirurgica Dissettiva e Operazioni Moderne Tecniche Fondamentali di Chirurgia Rinologica, Bruxelles, Novembre 2004.

A 21) 3rd Biennal Internationl instructional ”Milano Masterclass” Segment # 1 sinonasal & Skull base Endoscopic MicroSurgery. Milan, Italy. 4-6 Marzo 2005

A 22) 3rd Biennal Internationl instructional”Milano Masterclass” Segment # 2 Rhinoplsty & Pearls of Facial Plastic Surgery. Milan, Italy. 6-8 Marzo, 2005

A 23) Corso Pratico Intensivo di Anatomia Chirurgica Dissettiva e Operazioni Moderne Tecniche Fondamentali di Chirurgia Rinologica, Bruxelles Novembre 2005.

A 24) Advanced FESS dissection workshop at Academy of Athens foundation for medical reserch. Athens 18-20 Gennaio, 2006.

A 25) Corso Pratico Intensivo di Anatomia Chirurgica Dissettiva e Operazioni Moderne Tecniche Fondamentali di Chirurgia Rinologica, Bruxelles, Novembre 2006.

A 26) International Workshop on Micro-endoscopic Sinus Surgery. Università HNO-Klinik Mainz, Mainz Germany. 01-03, 2007.

A 27) Biennial International “Milano Masterclass” Sinonasal & Skull base Endoscopic MicroSurgery. Milan, Italy. 23-25 Marzo, 2007.

A 28) International Corse In Advanced Sinus Surgery Techinques. Amsterdam The Netherlands. 13-14 Marzo, 2008.

A 29) Minimally Invasive Endoscopic Surgery of the Cranial Base and Pituitary Fossa. Pittsburgh, USA. 1-3 Giugno, 2008.

A 30) Corso istruzionale. Anatomia chirurgica del naso e dei seni paranasali. SIO. Rimini, Italy. Maggio 2009

A 31) II Corso intensivo di dissezione chirurgica in Otorinolaringoiatria. Dissezione guidata step by step su cadavere. III segmento: orecchio. Ospedale San Raffaele Milan, Italy. 2009.

A 32) II Corso intensivo di dissezione chirurgica in Otorinolaringoiatria. Dissezione guidata step by step su cadavere. II segmento: ghiandole salivary. Ospedale San Raffaele Milan, 2009

A 33) Instructional course: An endoscopic approach to tumors of the infra-temporal fossa AAO-HNSF, Boston, Settembre 2010.

A 34) Instructional course: Endoscopic surgery for malignancies of sinonasal tract. AAOHNSF 2010, Settembre Boston, 2010

A 35) Instructional course: Sphenoid pathology and approaches to the sphenoid sinus. AAO-HNSF 2010, Settembre Boston

B) Relazioni a congressi

B 01) Convegno “Giornate nazionali Bormiesi di ORL”. Bormio 16-18 Gennaio 1997.Cisti aneurismatica ossea: case report. (**Trimarchi M**.)

B 02) Convegno “Aggiornamento sulla Chirurgia Endoscopica e Microscopica delle Cavità Naso-Sinusali”, Milano 1997: Rinosinusite: classificazione e indicazioni chirurgiche (Nicolai P., Tomenzoli D., Berlucchi M., Piazza C., **Trimarchi M**., Antonelli A.R.).

B 03) Corso di Perfezionamento in Rinologia, Pavia 1998: Papilloma invertito: trattamento endoscopico (Nicolai P., Tomenzoli D., Berlucchi M., **Trimarchi M**., Piazza C., Antonelli A.R.).

B 04) LXXXV Congresso S.I.O., Roma 1998: Il trattamento endoscopico del papilloma invertito naso-sinusale (Tomenzoli D., Nicolai P., Berlucchi M., Piazza C., **Trimarchi M.**).

B 05) II Croatian International Rhinosurgical Autumn School, Zagabria 1998: Endoscopic surgery for diffuse sinonasal polyposis. A report on 103 patients (Nicolai P., Tomenzoli D., Berlucchi M., **Trimarchi M**., Premoli G.).

B 06) I Congresso Nazionale della Società Italiana di Anatomia Patologica e Citologia Diagnostica, Taormina 1998: Lesioni distruttive sino-nasali e palatali da abuso inalatorio di cocaina. Quadro overlap con la granulomatosi di Wegener limitata?

(Manfredini C., **Trimarchi M**., Gregorini G., Majorana A., Morassi M.L., Nicolai P., Facchetti F.).

B 07) Advanced Endoscopic Surgery of the Rhinobasis and Sellar Region, Rozzano 1999: Endoscopic surgery for inverted papilloma. Experience on 19 patients (Tomenzoli D., Berlucchi M., **Trimarchi M**., Nicolai P.)

B 08) Congresso VII Giornate Nazionali Bormiesi di ORL, 2000. Un caso di distruzione nasale della linea mediana indotta dalla cocaina. (**Trimarchi M**., P. Nicolai, G. Gregorini, A.R. Antonelli).

B 09) XIII Congresso della Società Polispecialistica dei Giovani Chirurghi, Madonna di Campiglio 2000: Endoscopic treatment of sinonasal inverted papilloma (Tomenzoli D., Berlucchi M., **Trimarchi M**., Nicolai P., Antonelli A.R.).

B 10) XIII Congresso della Società Polispecialistica dei Giovani Chirurghi, Madonna di Campiglio 2000: The role of endoscopic surgery in the management of fungal sinusitis (Berlucchi M., Tomenzoli D., **Trimarchi M**., Nicolai P.).

B 11) Academy in ORL. Sinus Surgery: Endoscopic versus Open Surgery, Novara 2000: Il trattamento endoscopico della rinosinusite micotica (Nicolai P., Tomenzoli D., Berlucchi M., **Trimarchi M**., Lombardi D.)

B 12) Academy in ORL. Sinus Surgery: Endoscopic versus Open Surgery, Novara 2000: Il trattamento endoscopico del papilloma invertito naso-sinusale (Nicolai P., Tomenzoli D., Berlucchi M., **Trimarchi M**., Piazza C., Lombardi D.

B 13) XVIII Congress of European Rhinologic Society, Barcellona 2000: Clinical, histopathological, and Anca test findings in cocaine abusers with midline destructive lesions (**Trimarchi M.**, Nicolai P., Gregorini G., Facchetti F., Morassi L., Manfredini C., Russel K., Specks U.).

B 14) XVIII Congress of European Rhinologic Society, Barcellona 2000: Endoscopic surgery for sinonasal inverted papilloma: a debated approach (Tomenzoli D., Berlucchi M., **Trimarchi M**., Pagella F., Cerniglia M., Castelnuovo P., Nicolai P.).

B 15) XVIII Congress of European Rhinologic Society, Barcellona 2000: The role of endoscopy in revision surgery of the lacrymal system (Nicolai P., Puxeddu R., **Trimarchi M**., Serra A.) (su invito).

B 16) IX Congress of International Rhinologic Society, Washington 2000: Serologic and pathologic findings in patients with cocaine-induced midline destructive lesions: a comparison with Wegener granulomatosis (Nicolai P., **Trimarchi M**., Facchetti F., Specks U.).

B 17) IX Congress of International Rhinologic Society, Washington 2000: Clinical and radiologic findings in patients with cocaine-induced midline destructive lesions: a

comparison with Wegener granulomatosis (Nicolai P., **Trimarchi M**., Maroldi R., Gregorini G., Facchetti F., Russell K., Specks U., McDonald T.J.).

B 18) IX Congress of International Rhinologic Society, Washington 2000: Endoscopic treatment for sinonasal inverted papilloma: experience on 38 patients (Tomenzoli D., Berlucchi M., **Trimarchi M.**, Cerniglia M., Castelnuovo P., Nicolai P.).

B 19) Consensus Conference on Nasal Polyposis, Siena 2000: Postoperative outcome of frontal sinusitis in patients treated with endoscopic surgery for diffuse sinonasal polyposis (Nicolai P., Tomenzoli D., Berlucchi M., **Trimarchi M**., Lombardi D., Antonelli A.R.).

B 20) XVI Congresso Nazionale della Società Italiana di Otorinolaringologia Pediatrica, Saint Vincent 2000: La patologia ostruttiva delle vie aeree superiori da cause neoplastiche (Antonelli A.R., Nicolai P., Berlucchi M., Lombardi D., Tomenzoli D., **Trimarchi M**.).

B 21) 5th International Conference on Pediatric ORL, Graz 2001. Endoscopic Dacryocystorhinostomy (DCR) in the Pediatric Group. (Berlucchi M., **Trimarchi M**., Tomenzoli D., Staurenghi G., Nicolai P.).

B 22) 5th International Conference on Pediatric ORL, Graz 2001. Endoscopic Surgery for Juvenile Nasopharyngeal Angiofibroma in the Pediatric Group. (Nicolai P., Tomenzoli D., Berlucchi M., Cappiello J., **Trimarchi M**., Maroldi R.).

B 23) Combined Otolaryngological Spring Meetings. Boca Raton, 2002. Nasal Osteocartilagineous Necrosis in Cocaina abusers: Experience in 25 Patients. (**Trimarchi M**., Nicolai P., Lombardi D., Specks U.).

B 24) Combined Otolaryngological Spring Meetings. Bocaraton 2002. Endoscopic Surgery for Juvenile Angiofibroma (JA). How and when? (Nicolai P., Tomenzoli D., **Trimarchi M**., Lombardi D., Pianta L.).

B 25) XVIII IFOS World Congress 25-30 June 2005. Has apoptosis a role in the pathogenesis of Cocaine-induced midline destructive lesions? (CIMDL**). (M. Trimarchi,** A.Miluzio° P.C. Marchisio° M. Bussi).

B 26) American Rhinologic Society. Boca Raton, Florida 13-16 Maggio 2005. Cocaine-induced midline destructive lesions (CIMDL): cocaine concentration is more significant than time of exposure. **(M. Trimarchi**, M. Bussi, P. Nicolai*, M.L. Morassi*Miluzio°, P.C. Marchisio)

B 27) Corso Pratico Full-Immersion di Anatomia dissettiva e Tecniche Operatorie. Chirurgia Endoscopica Sinusale e basicranio- Livello avanzato- Bruxelles, 1-4 novembre 2006 Service d'Anatomie, Faculté de Médecine, ULB. Sessione di dissezione video-guidata: Cantotomia laterale. (**Trimarchi M.)**

B 28) 6th european congress of oto-rhino-laryngology head and neck surgery. EUFOS 2007. Cocaine-Induced Midline Destructive Lesions: A Comprehensive Overview. Key-note lecture. (**Trimarchi M.**) (su invito)

B 29) 95° Congresso Nazionale della Società Italiana di Otorinolaringologia e Chirurgia Cervico-Facciale, Torino 21-24 maggio 2008. Tavola rotonda Istituzionale. Lesioni centrofacciali indotte da abuso di cocaina: meccanismi eziopatogenetici e ricadute clinico terapeutiche. (M. Bussi , F. Palonta, **M. Trimarchi**).

B 30) American Rhinologic Society. Philadelphia 2009. Cocaine induced midline destructive lesions: an overview. Key-note lecture. (**Trimarchi M.**) (su invito)

B 31) ERS-ISIAN 2010. Geneva. Cocaine induced midline destructive lesions. Key-note lecture. (**Trimarchi M.**)

B 32) American Academy of-Head and Neck Surgery. Tavola rotonda “Granulomatous lesions of the nose and paranasal sinus” Boston, 2010. (**Trimarchi M.**) (su invito)

B 33) 4nd International Endoscopic Sinus Surgery Course, Kajetany, Varsavia, 2011. Key-note lecture. Medical therapy for CRS. Topical therapies. Pre- and postoperative medical treatment. (**Trimarchi M.**) (su invito)

B 34) 4nd International Endoscopic Sinus Surgery Course, Kajetany, Varsavia, 2011.

Key-note lecture. Endoscopic DCR. (**Trimarchi M.**) (su invito)

B 35) 4nd International Endoscopic Sinus Surgery Course, Kajetany, Varsavia, 2011.

Key-note lecture. Fungal Rhinosinusitis. (**Trimarchi M.**) (su invito)

B 36) 4nd International Endoscopic Sinus Surgery Course, Kajetany, Varsavia, 2011. Key-note lecture. Endoscopic Septoplasty and Surgical Management of the Inferior Turbinates. (**Trimarchi M.**) (su invito)

B 37) Combined European Otorhinolaryngology Head and Neck Surgery, Barcellona 2011. Cocaine abuse mimicking granulomatous disease. Keynote lecture. (**Trimarchi M.**) (su invito)

B 38) 5th World Congress for ENDOSCOPIC SURGERY of the BRAIN,SKULL BASE & SPINE and the First Global Update on FESS, THE SINUSES & THE NOSE 29 marzo-01 Aprile, 2012, Vienna Austria. Granulomatous diseases of the nose and paranasal sinuses. **M. Trimarchi**

B 39) 31 ° ISIAN, 17-21 June 2012, Tolosa, Francia. Endoscopic Septoplasty. **M Trimarchi**

B 40) American Academy of-Head and Neck Surgery, 9-12 Settembre, 2012, Washington, USA . Granulomatous diseases of the nose and paranasal sinuses. V.J.Lund, P. Batra, B. Wrobel, **M. Trimarchi**

B 41) 8° Corso Internazionale biennale Masterclass 20-24 Marzo 2013, Milano. cocaine induced midline destructive lesions. **M.Trimarchi**

B 42) 2nd Meeting Of European Academy Of Orl-Hns and Ceorl-Hns April 27-30, 2013, Nizza , France. Granulomatous diseases of the nose and paranasal sinuses. **M. Trimarchi**

B 43) American Academy of-Head and Neck Surgery, 29 Settembre-2 Ottobre, 2013, Vancouver, Canada. ISTRUCTIONAL COURSE: Granulomatous diseases of the nose and paranasal sinuses. P. Batra, B. Wrobel, **M. Trimarchi**

B 44) Third Bergamo Open Rhinoplasty course, 12-15 Marzo, 2014, Bergamo. Endoscopic Septoplasty. **M.Trimarchi.**

B 45) 6th World Congress for Endoscopic Surgery of the Brain, Skull Base & Spine, and Second Global Update on FESS, THE SINUSES & THE NOSE ENDOMILANO 2015. Istructional course IC SN 05: GRANULOMATOUS DISEASES OF THE NOSE AND PARANASAL SINUSES. dal 14-04-2014 al 14-04-2014. **M.Trimarchi.**

B 46) 101° Congresso Nazionale Della Società Italiana di Otorinolaringologia E Chirurgia Cervico-Facciale. May 28-31, 2014, Catania. Italia. **M.Trimarchi.**

B 47) 2° Congresso nazionale Accademia di Rinologia italiana, Catania. Lezione: "Lesione granulomatose del naso e dei seni paranasali". from 27 maggio 2014 **M.Trimarchi**.

B 48) International course “Extended endoscopic transnasal surgical approaches to the skull base. Laboratorio Anatomico “Head and Neck Surgery & Forensic Dissection Research center, HNS & fdrc” – A.O. Ospedale di Circolo e Fondazione Macchi, Università dell’Insubria. From 17 marzo 2015. **M.Trimarchi.**

B 49) L’endoscopia ad alta definizione con narrow band imaging nel trattamento delle neoplasie delle vads. Università' di Brescia. Lecture 'NBI nella valutazione del distretto nasosinusale e rinofaringeo" from 25-27 marzo, 2015. **M.Trimarchi.**

B 50) Corso di dissezione chirurgica nasosinusale, università' di brescia. Lecture: "DCR endoscopica" e Settoplastica endoscopica".from 15-01-2016 to 16-01-2016. **M. Trimarchi**

B 51) Rhinoplasty international dissection course, San Raffaele hospital, Milano. Faculty. Franz Wilhelm Baruffaldi Preis (Italy), Armando Boccieri (Italy), Mario Bussi (Italy), Nuri Celik (Turkey), Charles East (United Kingdom), Olivier Gerbault (France), Pier Giorgio Giacomini (Italy), Sebastian Haack (Germany), Milos Kovacevic

(Germany), Riccardo Mazzola (Italy), Lucia O. Piccioni (Italy), Enrico Robotti (Italy), **Matteo Trimarchi** (Italy) from 19 marzo, 2016.

B 52) 6th World Congress for Endoscopic Surgery of the skull base and brain

ENDOCHICAGO 2016. Granulomatous Diseases of The Nose and PNS

Moderator: Matteo Trimarchi, Panelists: **Matteo Trimarchi**, Sameep Kadakia, Valerie Lund, Bradley Otto from 15-05-2016 to 18-05-2016

B 53) Corso pratico avanzato sulla tecnica chirurgica funzionale endoscopica (fess) e rinosettoplastica su cadavere. Università degli Studi di Salerno, Dipartimento di Medicina, Chirurgia e Odontoiatra “Scuola Medica Salernitana”. lecture "Le vie lacrimali". from 06-04-2017 to 08-04-2017. **M. Trimarchi**

B 54) 104° Congresso Nazionale SIO – Sorrento 2017. Round table GLO: "La gestione della patologia delle vie lacrimali: diagnosi e varianti di trattamento" 24-27 maggio 2017: G.Trivella, M. Bignami, **M. Trimarchi.**

B 55) IFOS ENT World Congress 24 to 28 June 2017 - Paris. Round table (pag. 70 del programme) Non allergic rhinitis: what is it ? Chairman: T. BAUDOIN Moderator: S. LACROIX Panelists: T. Baudoin, E. De Corso, B. Landis, **M. Trimarchi**, D. Vicheva from 24-06-2017 to 28-06-2017

B 56) Istructional course a ifos ent World Congress- Paris. Instructional course (pag. 60 programme). Granulomatous diseases of the nose and paranasal sinuses Instructor: M. Trimarchi dal 24-06-2017 al 28-06-2017. **M. Trimarchi**

B 57) American Academy of-Head and Neck Surgery, 3- 6 Ottobre, 2019, Atlanta, U.S.A. Istructional course Granulomatous diseases of the nose and paranasal sinuses **M. Trimarchi**

B 58) 107° Congresso Nazionale SIO – MILANO 26 maggio, 2021. Round table: "La gestione della patologia delle vie lacrimali: diagnosi e varianti di trattamento" **M. Trimarchi**

B 59) 107° Congresso Nazionale SIO – MILANO 27 maggio, 2021. Round table: " Granulomatous diseases ANCA ASSOCIATED of the nose and paranasal sinuses" **M.Trimarchi**, U. Specks, B. Wrobel, P Batra.

B 59) American Academy of-Head and Neck Surgery 2 Ottobre 2021, Los Angeles U.S.A. Istructional course: Dacryocystorhinostomy: everything you need to know from diagnosis to surgery. **M. Trimarchi**

B 60) 108° Congresso Nazionale SIO – ROMA, 2021. ISTRUCTIONAL COURSE: "La gestione della patologia delle vie lacrimali: dalla diagnosi alla terapia" 25 maggio 2022. **M. Trimarchi**, A. Vinciguerra

B 61) 108° Congresso Nazionale SIO – ROMA, 2021. SIMPOSIO: UTILIZZO DEI CORTICOSTEROIDI SISTEMICI E LOCALI IN POLIPOSI NASALE, 26 maggio 2022. C. Pipolo, E. Cantone, V. Seccia, **M. Trimarchi**, E. De Corso

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C74) Vinciguerra A, Indelicato P, Giordano Resti A, Bussi M, **Trimarchi M**. Long- term results of a balloon-assisted endoscopic approach in failed dacryocystorhinostomies. Eur Arch Otorhinolaryngol. 2021 Jul 12:1–7. doi: 10.1007/s00405-021-06975-3. Epub ahead of print. PMID: 34251520; PMCID: PMC8273032.

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C 76) **Trimarchi M**, Vinciguerra A, Resti AG, Giordano L, Bussi M. Multidisciplinary approach to lacrimal system diseases. Acta Otorhinolaryngol Ital. 2021 Apr;41(Suppl. 1):S102-S107. doi: 10.14639/0392-100X-suppl.1-41-2021-10. PMID: 34060525; PMCID: PMC8172105.

C77) Vinciguerra A, Rampi A, Giordano Resti A, Barbieri D, Bussi M, **Trimarchi M**. Melanoma of the lacrimal drainage system: A systematic review. Head Neck. 2021 Jul;43(7):2240-2252. doi: 10.1002/hed.26705. Epub 2021 Apr 12. PMID: 33843111.

C78) **Trimarchi M**, Indelicato P, Vinciguerra A, Bussi M. Clinical efficacy of dupilumab in the treatment of severe chronic rhinosinusitis: The first case outside of a clinical trial. Clin Case Rep. 2021 Jan 16;9(3):1428-1432. doi: 10.1002/ccr3.3792. PMID: 33768860; PMCID: PMC7981721.

C79) Milardi G, Di Lorenzo B, Gerosa J, Barzaghi F, Di Matteo G, Omrani M, Jofra T, Merelli I, Barcella M, Filippini M, Conti A, Ferrua F, Pozzo Giuffrida F, Dionisio F, Rovere-Querini P, Marktel S, Assanelli A, Piemontese S, Brigida I, Zoccolillo M, Cirillo E, Giardino G, Danieli MG, Specchia F, Pacillo L, Di Cesare S, Giancotta C, Romano F, Matarese A, Chetta AA, **Trimarchi M**, Laurenzi A, De Pellegrin M, Darin S, Montin D, Marinoni M, Dellepiane RM, Sordi V, Lougaris V, Vacca A, Melzi R, Nano R, Azzari C, Bongiovanni L, Pignata C, Cancrini C, Plebani A, Piemonti L, Petrovas C, Di Micco R, Ponzoni M, Aiuti A, Cicalese MP, Fousteri G. Follicular helper T cell signature of replicative exhaustion, apoptosis, and senescence in common variable immunodeficiency. Eur J Immunol. 2022 May 13. doi: 10.1002/eji.202149480. Online ahead of print.PMID: 35562849

C80) Vinciguerra A, Rampi A, Yacoub MR, Tresoldi M, Tanzini U, Bussi M, **Trimarchi M**. Hypereosinophilia management in patients with type 2 chronic rhinosinusitis treated with dupilumab: preliminary results.Eur Arch Otorhinolaryngol. 2022 Apr 21. doi: 10.1007/s00405-022-07389-5. Online ahead of print.PMID: 35445858

C81) Giordano Resti A, Vinciguerra A, Bordato A, Rampi A, Tanzini U, Mattalia L, Bandello

F, **Trimarchi M** . The importance of clinical presentation on long-term outcomes of external dacryocystorhinostomies: Our experience on 245 cases. Eur J Ophthalmol. 2021 Nov 20:11206721211059702. doi: 10.1177/11206721211059702. Online ahead of print.PMID: 34806462

D) Libri

D1) Matteo Trimarchi, Paolo Capparà, Mario Bussi. Otorinolaringoiatria per l'odontoiatra. Casa Editrice Aries Due.

E) Capitoli di libro

E 01) Nicolai P., Berlucchi M., Piazza C., Tomenzoli D., **Trimarchi M**.: La anestesia locale nella chirurgia endoscopica naso-paranasale. In Piragine F.: Anestesia Locale in Otorinolaringoiatria. Stamperia e Legatoria Pisana, Pisa, 143-154, 1997.

E 02) A.R., Nicolai P., Berlucchi M., Lombardi D., Tomenzoli D., **Trimarchi M**.: La patologia ostruttiva delle vie aeree superiori da cause neoplastiche. In: L'Otorinolaringologia Pediatrica, Relazione Ufficiale Società Italiana di Otorinolaringologia Pediatrica "La Patologia Ostruttiva delle Vie Aeree Superiori", Roma, Vol. XI, 146-154, 2000.

E 03) Trimarchi M, Toma S, Pilolli F and Bussi M. Nasal Surgical Antomy. in Operative Cranial Neurosurgical Anatomy 2018. Thieme. Pag. 221-227.

E 04) Batra , PS, Wrobel B, Trimarchi M. Chapter 51. Systemic disease of the nose and sinuses in Cummings Otorhinolaryngology 7th e. Cummings Otolaryngology Head and Neck Surgery Seventh Edition. p. 788-794, Elsevier Inc, ISBN: 978-0-323-61179-4

E 05) Trimarchi M, Galli A, Teggi. ANCA-associated vasculitis - ENT Involvement. in Anti-Neutrophil Cytoplasmic Antibody (ANCA) Associated Vasculitis. p. 147-161, Springer International Publishing, ISBN: 978-3-030-02239-6, doi: 10.1007/978-3-030-02239-6

F) Sviluppo di Software

F 01) Rhinovision V 2.0. **M. Trimarchi**, D. Tomenzoli, B. Berlucchi, P. Nicolai, A.R. Antonelli 1997. System for archiving data and images in patients with inflammatory paranasal sinus pathologies.

F 02) NSNT ver 1.0 (Neoplasm of the sinonasal tract). V. Lund, **M. Trimarchi**, P. Nicolai, M.Pini, M. Senna 2001. System for archiving data and images in patients with inflammatory paranasal sinus pathologies.

G) Attività di reviwer

G 01) REVIEWER per la rivista "The European journal of Ophthalmology" dal 2006. dal 01-01-2006 a oggi

G02) REVIEWER per la rivista "The Laryngoscope" dal 2008. dal 01-01-2008 a oggi

G03) REVIEWER per la rivista "ACTA italica" dal 2016. dal 01-01-2014 a oggi

G04) REVIEWER per la rivista "PLOS" dal 2016 dal 01-01-2016 a oggi

H) Gruppi di ricerca internazionale

H 01) PARTECIPAZIONE A GRUPPO DI RICERCA INTERNAZIONALE. 1Gennaio 1999 fino a Novembre, 2001

Gruppo di ricerca caratterizzato dalla collaborazione internazionale fra il reparto di Thoracic Diseases Research Unit and Division of Pulmonary and Critical Care Medicine (KAR, US), Department of Otolaryngology (TJM), Mayo Clinic and Foundation, Rochester, Minnesota, USA e il reparto di otorinolaringoiatria, di nefrologia, anatomia patologia e radiologia dell'università di Brescia. Il gruppo di ricerca si è occupato di classificare gli aspetti peculiari nei pazienti con lesioni distruttive della linea mediana indotte dalia cocaina e nella granulaomatosi di Wegener (GPA). La collaborazione ha portato alla pubblicazione del seguente articolo:

Cocaine-induced midline destructive lesions: clinical, radiographic, histopathologic, and serologic features and their differentiation from Wegener granulomatosis. Trimarchi M, Gregorini G, Facchetti F, Morassi ML, Manfredini C, Maroldi R, Nicolai P, Russell KA, McDonald TJ, Specks U.
dal 01-01-1999 al 01-11-2001

H 02) PARTECIPAZIONE A GRUPPO DI RICERCA INTERNAZIONALE. 1Gennaio 1999 fino a Novembre, 2001

Gruppo di ricerca caratterizzato dalla collaborazione internazionale fra il reparto di Thoracic Diseases Research Unit and Division of Pulmonary and Critical Care Medicine (KAR, US), Mayo Clinic and Foundation, Rochester, Minnesota, USA e il reparto di otorinolaringoiatria dell'Univeristà vita Salute Ospedale san Raffaele e di nefrologia, dell'università di Brescia. Il gruppo di ricerca si è occupato di studiare le caratteristiche anticorpale come marker diagnostico nei pazienti con lesioni distruttive della linea mediana indotte dalia cocaine. La collaborazione ha portato alla pubblicazione del seguente articolo.

Antineutrophil cytoplasmic antibodies reacting with human neutrophil elastase as a diagnostic marker for cocaine-induced midline destructive lesions but not autoimmune vasculitis. Wiesner O, Russell KA, Lee AS, Jenne DE, Trimarchi M, Gregorini G, Specks U.

H 03) PARTECIPAZIONE A GRUPPO DI RICERCA INTERNAZIONALE 1Gennaio 2000 fino a Ottobre 2004.

The Neoplasms of the Sinonasal Tract software package (NSNT v 1.0) è il risultato della collaborazione fra il reparto di Otorinolaringoiatria dell'Ospedale San Raffaele di Milano con the Institute of laryngology and Otology, The Royal National Throat, Nose and EAR Hospital a Londra, il reparto di Computer Science, Università di Pavia e la clinica di Otorinolaringoiatria, Università di Brescia .
Il database si pone l'obiettivo di standardizzare la raccolta dei dati in pazienti con neoplasie del naso e dei seni paranasali per poter facilitare e migliorare l'analisi multicentrica di tumori molto aggressive ma rari. La collaborazione ha portato alla pubblicazione di :

Database for the collection and analysis of clinical data and images of neoplasms of the sinonasal tract. Trimarchi M, Lund VJ, Nicolai P, Pini M, Senna M, Howard DJ. Ann Otol Rhinol Laryngol. 2004 Apr;113(4):335-7.
dal 01-01-2002 al 01-01-2004

H 04) PARTECIPAZIONE A GRUPPO DI RICERCA INTERNAZIONALE dal gennaio 2010 fino al dicembre 2013.
European Rhinologic Society Advisory Board on Endoscopic Techniques in the Management of Nose, Paranasal Sinus and Skull Base Tumours.
Ha portato alla seguente pubblicazione:

European position paper on endoscopic management of tumours of the nose, paranasal sinuses and skull base. Rhinol supplement 2010 Jun 1,21:1-143.Lund VJ, Stammberger H, Nicolai P, Castelnuovo P, Beal T, Beham A, Bernal-Sprekelsen M, Braun H, Cappabianca P, Carrau R, Cavallo L, Clarici G, Draf W, Esposito F, Fernandez-Miranda J, Fokkens W, Gardner P, Gellner V,Hellquist H,

Hermann P, Hosemann W, Howard D, Jones N, Jorissen M, Kassam A, Kelly D, Kurschel-Lackner S,Leong S, McLaughlin N, Maroldi R, Minovi A, Mokry M, Onerci M, Ong YK, Prevedello D, Saleh H, Sehti DS, Simmen D, Snyderman C, Solares A, Spittle M, Stamm A, Tomazic P, **TRIMARCHI M,** Unger F, Wormald PJ, Zanation A;

H 05) PARTECIPAZIONE A GRUPPO DI RICERCA INTERNAZIONALE dal 9 Gennaio, al 1 giugno, 2011

Participation in the research activity for prpviding guidelines for diagnosis, treatment and rehabilitation of persons dependent on cocaine of the Department of Anti-Drug Policies of the Presidency of the Council of Ministers. ZEROCOCA - Recommendations for the diagnosis, treatment and rehabilitation of people dependent on cocaine: Serpelloni G, Randazzo L, Marino V. Agus A., S.O.C. Dipendenze Ser.T. Monfalcone Ass2 "Isontina", Gorizia. Alessandrini F., Neuroradiology Service, Azienda Ospedaliera Universitaria Integrata di Borgo Trento, Verona. Mazzoleni M., Centro Antiveleni di Pavia, National Toxicological Information Centre, Ospedale Istituto Scientifico di Pavia, IRCCS Fondazione Salvatore Maugeri, Pavia. Minetti T., Department of Pathological Dependencies C. Olievenstein, ASL To2 Turin. Nava F., ULSS 16 Padua. Pani P., Director, ASL Cagliari. Petrolini V., Poison Center of Pavia, National Centre of Toxicological Information, Scientific Institute of Pavia, IRCCS Fondazione Salvatore Maugeri, Pavia. Randazzo L., Department of Dependencies, ASL Varese. Rimondo C., Department of Anti-Drug Policies, Presidency of the Council of Ministers, Minister for International Cooperation and Integration, Rome. Rognoni C., Poison Centre of Pavia, Presidency of the Council of Ministers, National Centre for Toxicological Information, Ospedale Istituto Scientifico di Pavia, IRCCS Fondazione Salvatore Maugeri, Pavia. Schifano F., University of Hertfordshire, Department of Clinical Pharmacology. Serpelloni G., Department of Anti-Drug Policies, Presidency of the Council of Ministers, Minister for International Cooperation and Integration, Rome. Somaini L., Department of Dependency Pathology, Drug and Alcohol Addiction Service, Cossato, ASL Biella. Tischer MC.,

Paeditrician, ASL Varese, Saronno. **TRIMARCHI M**., Unit of Otorhinolaryngology, IRCCS San Raffaele, Milan.

H 06) PARTECIPAZIONE A GRUPPO DI RICERCA INTERNAZIONALE dal 20 gennaio, 2011 al 3 maggio 2012 responsible for studies and scientific research for the Department of Anti-Drug Policies of the Presidency of the Council of Ministers for implementation of recommendations for ENT specialists on cocaine users. La collaborazione ha portato alla pubblicazione del volume

Use of cocaine and destructive facial injuries: guidelines for ENT specialists" 25 May 2011 Text prepared by: Bussi M., **Trimarchi M**., Serpelloni G., Rimondo C., Technical superviion: Piero Nicolai, Paolo Castelnuovo, Marco De Vincentiis, Andrea Gallo, Antonio Pastore, Roberto Puxeddu, Stefano Sellari Franceschini, Riccardo Speciale.

H 07) Dal 1 gennaio 2011 al 25 maggio, 2015
Scientific responsible; grant for Genetic analysis of patients with cocaine induced midline destructive lesions (CIMDL) through exome sequencing funding agency. Presidency of the Council of Ministers, Department of AntiDrug Policies, Rome. Questo finanziamento ha portato alla pubblicazione di:

Trimarchi M, Bertazzoni G, Vinciguerra A, Pardini C, Simeoni F, Cittaro D, Bussi M, Lazarevic D. Gene Expression Analysis in Patients with Cocaine-Induced Midline Destructive Lesions. Medicina (Kaunas). 2021 Aug 24;57(9):861. doi: 10.3390/medicina57090861. PMID: 34577784; PMCID: PMC8469603.

4. ATTIVITA' DI INSEGNAMENTO

Dal 2002 ha il ruolo di tutor in otorinolaringoiatria alla scuola di medicina dell'Università Vita Salute San Raffaele in Milano,

2002/2003

Esercitazioni di Clinica Otorinolaringoiatrica nel corso integrato di chirurgia agli studenti del Corso di Laurea in Medicina e Chirurgia (Università Vita Salute, Fondazione San Raffaele). Stesura di tesi di laurea, lezioni per il Corso di Clinica Otorinolaringoiatrica in sostituzione del Titolare, partecipazione a commissioni di esami.

2003/2004

Esercitazioni di Clinica Otorinolaringoiatrica nel corso integrato di chirurgia agli studenti del Corso di Laurea in Medicina e Chirurgia (Università Vita Salute, Fondazione San Raffaele). Stesura di tesi di laurea, lezioni per il Corso di Clinica Otorinolaringoiatrica in sostituzione del Titolare, partecipazione a commissioni di esami.

2004/2005

Esercitazioni di Clinica Otorinolaringoiatrica nel corso integrato di chirurgia agli studenti del Corso di Laurea in Medicina e Chirurgia (Università Vita Salute, Fondazione San Raffaele). Stesura di tesi di laurea, lezioni per il Corso di Clinica Otorinolaringoiatrica in sostituzione del Titolare, partecipazione a commissioni di esami. Collaborazione nell'esecuzione del corso elettivo "Otorinolaringoiatria: le sette cose che non farò da grande" destinato agli studenti dal quarto anno in poi nel Corso di Laurea Specialistica in Medicina e Chirurgia Docente della Scuola di Specializzazione in Otorinolaringoiatria della Facoltà di Medicina e Chirurgia dell'Università Vita Salute di Milano per gli insegnamenti di Statistica medica (MED01) e Otorinolaringoiatria (MED31) (Rinologia)

2005/2006

Esercitazioni di Clinica Otorinolaringoiatrica nel corso integrato di chirurgia agli studenti del Corso di Laurea in Medicina e Chirurgia (Università Vita Salute, Fondazione San Raffaele). Stesura di tesi di laurea, lezioni per il Corso di Clinica Otorinolaringoiatrica in sostituzione del Titolare, partecipazione a commissioni di esami.

2006/2007

Esercitazioni di Clinica Otorinolaringoiatrica agli studenti del Corso di Laurea in Medicina e Chirurgia (Università Vita Salute, Fondazione San Raffaele). Stesura di tesi di laurea, lezioni per il Corso di Clinica Otorinolaringoiatrica in sostituzione del Titolare, partecipazione a commissioni di esami. Collaborazione nell'esecuzione del corso elettivo "L'Oncologia Head & Neck: possibilità di intervento per un buon medico ed un grande Istituto" destinato agli studenti del quarto anno nel Corso di Laurea Specialistica in Medicina e Chirurgia.

2007/2008

Tutor per gli insegnamenti Otorinolaringoiatrici nell'ambito dell attività Professionalizzanti (APRO) in Chirurgia e Specializzazioni Chirurgiche rivolte agli studenti del quinto anno del Corso di Laurea Specialistica in Medicina e Chirurgia. del Corso di Laurea in Medicina e Chirurgia Università Vita Salute, Fondazione San Raffaele. Stesura di tesi di laurea, lezioni per il Corso di Clinica Otorinolaringoiatrica in sostituzione del Titolare, partecipazione a commissioni di esami. Collaborazione nell'esecuzione del corso elettivo "L'Oncologia Head & Neck: possibilità di intervento per un buon medico ed un grande Istituto" destinato agli studenti del quarto anno nel Corso di Laurea Specialistica in Medicina e Chirurgia

2007-ad oggi

Courses in Medical Statistics Otorhinolaryngology (Rhinology) in the Specialization Programme in Otorhinolaryngology (University Vita Salute, San Raffaele).

Responsible for clinical rounds in Otorhinolaryngology in the Medical School at the University Vita Salute, San Raffaele. Participated in thesis and exam commissions, and the course in Otorhinolaryngology.

2013/2014

Collabora all'attività didattica presso il Corso di Laurea magistrale in Odontoiatria e Protesi Dentaria per il Corso Integrato "Scienze mediche I" con il prof Mario Bussi, Coordinatore del Corso, come docente su invito svolgendo 10 ore di lezione al Corso integrato di Scienze mediche I, Otorinolaringoiatria – S.S.D. MED/31 - 16 ore di didattica frontale.

2014/2015

Incarico di Insegnamento presso il Corso di Laurea magistrale in Odontoiatria e Protesi Dentaria per SSD MED/31 Chirurgia Generale tramite affidamento diretto del corso integrato di “Scienze Chirurgiche“ per 20 ore di Chirurgia Odontostomatologica.

2015- ad oggi

Attività didattiche in qualità di Professore associato in otorinolaringoiatria per la Scuola di Specializzazione in Otorinolaringoiatria, Università Vita-Salute San Raffaele di Milano, Settore scientifico disciplinare MED 01–MED 31. 10 ore

Insegnamento presso il Corso di Laurea magistrale in Odontoiatria e Protesi Dentaria Otorinolaringoiatria, Università Vita-Salute San Raffaele di Milano, Settore scientifico disciplinare MED 01–MED 31, 46 ore

Insegnamento presso il Corso di Laurea magistrale di medicina e chirurgia, settore scientifico disciplinare MED 01–MED 31, 4 ore.

Insegnamento presso il Corso di Laurea magistrale di medicina e chirurgia, MD programme in inglese, settore scientifico disciplinare MED 01–MED 31, 8 ore.

Responsabilita' di studi e ricerche scientifiche affidati da qualificate istituzioni pubbliche o private

RESPONSABILITÀ' DI STUDI E RICERCA SCIENTIFICA AFFIDATA dal Dipartimento Politiche Antidroga della Presidenza del Consiglio dei Ministri per la realizzazione delle linee di indirizzo per gli specialisti otorinolaringoiatri per i pazienti cocainomani.
Lo studio ha portato alla realizzazione del volume "Uso di cocaina e lesioni distruttive facciali: linee di indirizzo per gli specialisti otorinolaringoiatri" 25 maggio 2011 Testo preparato a cura di: Bussi M., Trimarchi M., Serpelloni G., Rimondo C., Supervisione tecnica: Piero Nicolai, Paolo Castelnuovo, Marco De Vincentiis, Andrea Gallo, Antonio Pastore, Roberto Puxeddu, Stefano Sellari Franceschini, Riccardo Speciale

RESPONSABILE SCIENTIFICO GRANT

Analisi genetica di pazienti affetti da lesioni distruttive della linea mediana indotte da Cocaina (CIMDL) tramite exome sequencing

FUNDING AGENCY

Presidenza del Consiglio dei Ministri, Dipartimento Politiche Antidroga, Roma dal 20-12-2013 al 28-02-2016

5. ORGANIZZAZIONE DI CONGRESSI

1) Update on Wegener's granulomatosis upper and lower respiratory tract. Università di Brescia. Settembre 28, 2001.

2) Primo corso intensivo di clinica e anatomia chirurgica. Patologia oncologica delle ghiandole salivari maggiori. Novembre 10-12- 2002. Milano

3) Wegener granulomatosis a multidisciplinary approach "Updating di Otorhinolaryngology in San Raffaele" Marzo 27, 2003. Milano. .

4) Surgery on live. Endoscopic sinonasal surgery & Rhinoplasty 2004. 29 Giugno- 2 luglio, Milano.

5) La chirurgia di recupero dei carcinomi laringei radiotrattati. 2 -3 Febbraio 2006. Milano.

6) Wegener's granulomatosis 2° Otorhinolaringology Workshop. Università Vita Salute San Raffaele Milano. 12 Marzo, 2007.

7) II Corso intensivo di dissezione chirurgica in Otorinolaringoiatria. Dissezione guidata step-by-step su cadavere. 24-27 Novembre, 2009. Milano

8) III Corso intensivo di dissezione chirurgica in Otorinolaringoiatria. Dissezione guidata step-by-step su cadavere. 21-27 Novembre, 2010. Milano

9) IV Corso intensivo di dissezione chirurgica in Otorinolaringoiatria. Dissezione guidata step-by-step su cadavere. 20-25 Novembre, 2011. Milan, Italy

10) What's new in rhinology Faculty: V.J. Lund, PJ. Wormald, M. Bussi, P. Nicolai, P. Castelnuovo, R. Maroldi, E. Pasquini, M. Trimarchi. 4 Settembre, 2012, Milano, Itay

11) Sino nasal & Skull Base Dissection course: extended endoscopic transnasal surgical approches to the Skull Base. Milano, 12 – 13 Aprile, 2014

12) IX Convegno Annuale GLO, Milano, 23 marzo 2019.

13) X Convegno Annuale GLO, Milano, 1-2 luglio, 2021.

14) 107° Congresso Nazionale SIOeChCF, Milan, 26-27-28-29 Maggio, 2021.

15) Corso intensivo di dissezione chirurgica in Otorinolaringoiatria. Dissezione guidata step-by-step su cadavere. 5-6 Maggio 2022, Milano.

6. CASISTICA OPERATORIA

DAL 2002 al 2022, ha eseguito più di 6000 interventi nell'ambito testa e collo di cui più di 5000 come primo chirurgo.

Interventi chirurgici del naso e dei seni paranasali in anestesia generale (chirurgia endoscopica funzionale del naso e dei seni paranasali, poliposi nasale, settoplastica, rinosettoplastica).

Chirurgia avanzata del naso e dei seni paranasali e della base cranica (asportazione di tumori benigni e maligni, nasofaringectomie endoscopiche, plastiche della base cranica, in collaborazione con il Dipartimento di Neurochirurgia)

Interventi chirurgici in microlaringoscopia diretta sulla laringe e sulle corde vocali (biopsie, cordectomie laser assistite, asportazione di lesioni glottiche/sopraglottiche)

Chirurgia oncologica della testa e del collo (tracheotomie, maxillectomie radicaliche transfacciali, tiroidectomie, parotidectomie superficiali e totali, svuotamento linfonodale del collo, laringectomie totali, sialectomie sottomandibolari, glossectomie transorali).

Chirurgia ORL pediatrica (adenoidectomie, tonsillectomie, asportazione di cisti del dotto tireoglosso, asportazione di granuli e lesioni del cavo orale, interventi sul frenulo linguale).

Interventi chirurgici ambulatoriali in anestesia locale (biopsie ed asportazione di lesioni del naso, rinofaringe e cavo orale; turbinoplastica laser; polipectomie nasali endoscopiche; interventi di radiofrequenza sul palato molle per russamento.

7. RIASSUNTO DELL'ATTIVITA' SCIENTIFICA

The scientific activity of prof. Matteo Trimarchi can be grouped into several main areas: rhinology, laryngology and neck surgery, vestibology and radiology.

1- Rinologia

In collaborazione con gli Istituti di Nefrologia, Anatomia Patologica, Radiologia e Odontoiatria dell'Università di Brescia e con la Thoracic Diseases Research Unit della Mayo Clinic sono stati analizzati due gruppo di pazienti, uno con lesioni distruttive centro-facciali da abuso di cocaina e uno con GPA. Il dato più rilevante è risultato essere la positività in un numero elevato di pazienti cocainomani del test per verificare gli anticorpi anti citoplasma dei neutrofili (ANCA); in 4 di essi, addirittura, il pattern di positività era del tutto indistinguibile da quello della GPA. Tuttavia, la positività ANCA dei pazienti con abuso di cocaina presentava alcune caratteristiche atipiche rispetto a quella dei pazienti con GPA: prevalenza del pattern perinucleare-ANCA (P-ANCA), associazione del pattern P-ANCA con proteinasi3-ANCA (PR3-ANCA) e completa assenza di mieloperossidasi-ANCA (MPO-ANCA) malgrado l'elevata prevalenza di pattern P-ANCA.

In collaborazione con la Mayo Clinic è stato possibile identificare dei pattern ANCA tipici nei pazienti cocainomani. E' stata identificata la presenza di elastasi-ANCA (HNE-ANCA) nei pazienti che abusano di cocaina che invece è risultato essere assente in un gruppo di controllo di 604 pazienti. Gli HNE-ANCA possono discriminare i pazienti cocainomani da quelli affetti da granulomatosi di di Wegener mentre i test convenzionali non sono in grado. L'apoptosi sembra essere uno dei processi implicati nella patogenesi delle lesioni distruttive della linea mediana. Le cellule in cultura con la cocaina presentano un aumento dell'apoptosi che è dose e tempo dipendente. Dalla valutazione dei preparati istologici in 60 pazienti (30 pazienti cocainomani,10 pazienti sani, 10 pazienti con poliposi nasale e 10 con GPA) è stato dimostrato che l'apoptosi è frequente nelle cellule nasali dei pazienti cocainomani mentre non è presente in tutti gli altri valutati. Gli ANCA che legano l'elastasi (NE)e la proteinasi 3 (PR3) sono identificabili in molti pazienti cocainomani ma l'eziopatogenesi e la specificità di questi anticorpi non sono noti. Questo studio in collaborazione con la Thoracic Diseases Research Unit della Mayo Clinic di Rochester è stato condotto per verificare l'effetto dell'elastasi ANCA sull'attività enzimatica dell'elastasi e per determinare se questi anticorpi interferiscono con l'effetto fisiologico dell'inibitore della secrezione delle leucoproteasi (SLPI) e per valutare la specificità dell'antigene per entrambi, NE e PR3 ANCA nei pazienti cocainomani. In conclusione gli effetti funzionali degli NE

ANCA sull'attività enzimatica del SPLI non possono essere implicati nella patogenesi dei CIMDL. La risposta degli ANCA includendo la reattività per PR3 ANCA nei pazienti con CIMDL è diversa da quella descritta nei pazienti con granulamatosi di Wegener.
Nel 2012 sono state pubblicate 2 revisioni della letteratura su invito della rivista Autoimmunity Reviews, una sulla granulomatosi di Wegener e una sulle lesioni indotta dalla cocaina.
Un altro campo di studio è stato quello della patologia delle vie lacrimali. Il trattamento endoscopico è stato utilizzato nella chirurgia di revisione dei fallimenti di dacriocistorinostomia per via esterna. I risultati ottenuti confermano il vantaggio della tecnica endonasale che, oltre ad essere mini-invasiva, consente di identificare e correggere le anomalie anatomiche e le alterazioni flogistiche croniche naso-paranasali spesso associate ad una stenosi delle vie lacrimali. La tecnica endoscopica è stata applicata anche per il trattamento di 88 pazienti con ostruzione delle vie lacrimali utilizzando un anastomosi tra il un lembo di mucosa nasale con quello del sacco lacrimale. Continuando questa chirurgia siamo arrivati ad analizzare i dati su 500 pazienti e abbiamo scritto le alcune delle linee guida per la gestione di questa patologia.
La tecnica endoscopica è stata usata con successo anche per il trattamento dell' angiofibroma rinofaringeo e del papilloma invertito. Per il primo i criteri di inclusione utilizzati per il trattamento endoscopico sono stati il risultato ottimale della embolizzazione, la minima vascolarizzazione dal sistema della carotide interna e la limitata estensione alle sedi anatomiche adiacenti (Stadio I-II secondo la classificazione di Fisch). Per quanto attiene il papilloma invertito, sono stati giudicati non passibili di trattamento endoscopico i pazienti con massiva erosione del basicranio, diffuso interessamento del seno frontale, invasione intracranica o intraorbitaria, o con importanti esiti cicatriziali da pregressa chirurgia. Nel gruppo di 21 pazienti operati per papilloma invertito non si sono osservate recidive mentre una sola persistenza è stata riscontrata nel gruppo di 15 pazienti trattati per angiofibroma.

In collaborazione con il Reparto di Urologia dell'Ospedale San Raffaele di Milano è stato studiato l'effetto del Sildenafil (Viagra), un inibitore della fosfodiesterasi tipo 5 approvato per il trattamento della disfunzione erettile. Sebbene questo farmaco sia ben tollerato, è associato ad effetti collaterali, quali cefalea, disturbi visivi, dispepsia e congestione nasale. L'effetto del Viagra è stato valutato nel distretto nasale in uno studio doppio cieco in 11 pazienti sani senza disturbi respiratori. Tutti i pazienti sono stati sottoposti a valutazione dei parametri basali, rinomanometria acustica ed endoscopia nasale prima e dopo il placebo o Sildenafil con stimolazione sessuale visiva. Per lo studio dei dati è stato utilizzato il test di

Kruskal–Wallis. Dopo somministrazione del Sildenafil l'area MCA e il VOL presentavano valori più bassi rispetto a quelli osservati con il placebo. Inoltre l'endoscopia nasale ha mostrato un aumento di volume del turbinato inferiore con una differenza oggettiva rispetto al placebo. Questo studio preliminare ha dimostrato che il Sildenafil unitamente alla stimolazione sessuale riduce considerevolmente il volume nasale.

In collaborazione con l'Institute of Laryngology and Otology, Royal National Throat, Nose and Ear Hospital, London, England, è stato sviluppato un software per l'archiviazione dei dati dei pazienti con Tumori dei seni paranasali. Il software (NSNT v 1.0) fornsice una database per i pazienti con neoplasie dei seni paranasali, facilitando la standardizzazione dei dati e dell'analisi statistica. Questo software è rimasto a disposizione gratuita in rete sul sito della rivista Annals of Otology Rhinology and Laryngology per tutto l'anno 2004.

In collaborazione con l'International and European Rhinologic Societies Advisory Board on Endoscopic Techniques in the management of Nose, Paranasal Sinus and Skull Base Tumors ha contribuito alla stesura della posizione europea sul trattamento dei tumori del naso dei seni paranasali e della base cranica.

Ha ottenuto un finanziamento dalla Presidenza del Consiglio dal Dipartimento antidroga per lo studio della genetica nei pazienti con lesione distruttive della linea mediana indotte dalla cocaina

2 - **Laringologia e chirurgia del collo**

Il termine di "leucoplachia" laringea ha un significato eminentemente clinico e ad essa possono corrispondere differenti quadri istologici a comportamento biologico variabile. Requisito essenziale per una corretta classificazione istologica è che la lesione sia asportata "en bloc" in tessuto sano ed esaminata in sezioni seriate. In ogni trattamento è stata utilizzata intraoperatoriamente la colorazione vitale con blu di toluidina al 2% per demarcare macroscopicamente i limiti di escissione della lesione. La diagnosi istologica fu la seguente: iperplasia (4 casi), acantosi (4 casi), cheratosi (5 casi), paracheratosi (1 caso), laryngeal intraepithelial neoplasia (LIN) I (1caso), LIN II (1 caso), LIN III (4 casi), carcinoma squamoso (20 casi), carcinoma verrucoso (3 casi). Il laser CO2 si è dimostrato un mezzo chirurgico estremamente utile e vantaggioso nell'esecuzione delle biopsie escissionali, sia a

fini diagnostici che terapeutici. Il trattamento del collo nei carcinomi delle prime vie aerodigestive è ancora un problema controverso anche se oggi c'è una tendenza in letteratura verso la chirurgia nei colli N0 quando la probabilità di metastasi linfonodali è maggiore del 20%. Nella chirurgia d'elezione ogni sforzo è teso a conservare le strutture linfatiche non interessate in un collo positivo. La prevalenza di linfonodi metastatici e la loro distribuzione nei vari livelli del collo è stata valutata in una corte di 402 pazienti consecutivi trattati per carcinoma squamocellulare sopraglottico per definire una strategia nel trattamento del collo. La conclusione indica che uno svuotamento elettivo latero cervicale (Livelli II-IV) è raccomandato nei carcinomi sopraglottici T2-T4 N0; la chirurgia in entrambe i lati è indicata nei casi in cui la lesione non è strettamente laterale. Qualora ci sia una positività clinica, radiologica o un evidenza intraoperatoria a qualsiasi livello effettuiamo uno svuotamento selettivo II-V.

La telechirurgia robotica è un nuovo concetto nell'assistenza chirurgica che ha acquisito rilevanza negli ultimi due decenni. Con l'introduzione delle reti mobili 5G, questo concetto sta diventando pratico. Qui, riportiamo è stato eseguito con successo un intervento di microchirurgia laser transorale sulle corde vocali di un cadavere umano adulto situato a 15 km dalla sede del primo chirurgo. Ciò è stato possibile grazie a un sistema di telecomunicazioni 5G ad alta larghezza di banda e bassissima latenza, che ha consentito il controllo remoto preciso degli strumenti robotici e la visualizzazione 3D full HD (1920×1280 pixel) del sito chirurgico. I nostri risultati dimostrano che le competenze chirurgiche possono essere sfruttate e condivise in modo efficiente utilizzando il nuovo standard di telecomunicazione 5G. Questo è fondamentale per operazioni altamente specializzate come la microchirurgia qui dimostrata, che richiedono competenze chirurgiche non ampiamente disponibili. Pertanto, la possibilità di operare a distanza può portare notevoli benefici ai sistemi sanitari, riducendo i costi e migliorando la qualità delle cure per i pazienti. Inoltre, rende il telementoring una realtà, consentendo a chirurghi esperti di essere presenti virtualmente in più sale operatorie per guidare e formare i colleghi meno esperti

3 - Vestibologia

Abbiamo studiato l'effetto delle soluzioni ipotoniche e ipertoniche (il valore normale era 240 mOsm) in un labirinto di rana isolato. Le soluzioni ipotoniche (60-180 mOsm) sono state ottenute riducendo il contenuto perilinfatico di NaCl. Le soluzioni ipertoniche (300-420 mOsm) sono state ottenute aggiungendo a una soluzione perilinfatica normale NaCl,

glucosio, saccarosio, glicerolo, mannitolo e urea. I risultati hanno dimostrato che ogni tipo di attività recettoriale è inibita da una soluzione ipotonica. Al contrario una soluzione ipertonica produce diversi effetti sulla attività spontanea ed evocata. L'attività spontanea, eccetto che per l'urea, è costantemente aumentata mentre quella evocata è costantemente ridotta dalle soluzioni ipertoniche. Lo studio di variazione dell'osmolarità nei preparati isolati di canali semocircolari può dare dei dati importanti nello studio della malattia di Meniere.
Il postnistagmo otticocinetico (PNOC) può essere considerato una reazione postuma ("after discharge") di strutture nervose centrali in precedenza attivate da stimoli otticocinetici. In un gruppo di 28 soggetti normali d'ambo i sessi di età compresa tra 18 e 28 anni è stata condotta un'indagine per lo studio del PNOC evocato sia sul piano orizzontale che sul piano verticale. I valori massimi della frequenza e dell'ampiezza del PNOC evocato sia sul piano orizzontale che su quello verticale, si notarono nei primi 30 secondi della reazione postnistagmica. La latenza media del PNOC era superiore dopo stimolazione verticale, mentre la durata media della reazione postnistagmica era superiore dopo stimolazione orizzontale.

4 Radiologia Otorinolaringoiatrica

La stretta collaborazione con i Colleghi dell'Istituto di Radiologia dell'Università di Brescia ha consentito l'approfondimento delle caratteristiche radiologiche nei pazienti con lesione distruttive della linea mediana e con vasculite.

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Cocaine-Induced Midline Destructive Lesions

Clinical, Radiographic, Histopathologic, and Serologic Features and their Differentiation from Wegener Granulomatosis

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Introduction

Habitual nasal insufflation of pulverized cocaine may cause mucosal lesions. Mild changes cause nasal stuffiness, headache, or hyposmia. If cocaine use becomes chronic and compulsive, progressive damage of the mucosa and perichondrium leads to ischemic necrosis of the septal cartilage and perforation of the nasal septum. Occasionally, cocaine-induced lesions cause extensive destruction of the osteocartilaginous structures of the nose, sinuses, and palate that mimics the clinical picture of other diseases associated with necrotizing midfacial lesions (1, 4, 17, 20, 31, 38, 43, 49, 58, 62, 63, 66, 74). The mucosal damage induced by cocaine is multifactorial. The vasoconstrictive effect of the drug is thought to be the most important factor (8, 17, 20, 38). However, the irritant effect of adulterants of the drug, the traumatic effect on the mucosa caused by cocaine crystals insufflated at high velocity, and recurrent nasal infections all seem to contribute to chronic tissue destruction (20, 38). Progressive nasal obstruction, epistaxis with crusting, and ulceration of the nasal mucosa with or without septal perforation are also characteristic manifestations of nasal involvement by Wegener granulomatosis (WG). The differentiation of cocaineinduced midline destructive lesions (CIMDL) and limited WG may be difficult, particularly if the patients do not readily admit to their substance abuse.

Antineutrophilic cytoplasmic antibodies (ANCA) directed against proteinase 3 (PR3) or myeloperoxidase (MPO) are sensitive and specific markers for the idiopathic small vessel vasculitides including WG (30). It is generally believed that the presence of a positive ANCA test result with either of the 2 antigen specificities facilitates the differential diagnosis of WG. However, instances of positive ANCA test results have been reported in patients with lesions attributed to cocaine abuse (4, 29). We found positive ANCA test results in an unexpectedly large proportion of patients with CIMDL. In several instances their lesions were clinically indistinguishable from WG limited to the upper respiratory tract (28). This seems to limit the usefulness of routine ANCA testing for the unequivocal differentiation of cocaineinduced nasal lesions from limited WG.

We performed the present study to identify clinical, radiographic, and histopathologic features that allow the distinction of patients with severe CIMDL from those with WG and to further characterize their ANCA.

Patients and Methods

Patients

Between January 1991 and December 1999, 18 cocaine abusers with midline destructive lesions were evaluated in the Department of Otolaryngology of the University of Brescia, Italy (Table 1). The patients ranged in age from 22 to 66 years (median, 37 yr). Ten were men and 8 women. The follow-up period ranged from 12 to 108 months (median, 34 mo). At the time of first observation, all patients except 1 admitted to cocaine use. In 7 patients reliable information on abuse duration and dose could not be obtained, 2 patients had a history of abuse with an undetermined dose lasting 6 and 8 years, respectively. One patient had been using 1–3 g per week irregularly. The remaining 8 patients had been using cocaine for 2–30 years, at a dose ranging from 1 to 15 g per week.

The control population consisted of all 21 consecutive WG patients who underwent a nasal biopsy in the Department of Otolaryngology of the University of Brescia during the same time frame (see Table 1). Patients' ages ranged from 30 to 64 years (median, 45 yr). Nine were men and 12 women. The follow-up

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period **TABLE 1. Clinical features and laboratory values at presentation of patients with CIMDL and WG**

	CIMDL (n 18)	WG (n 21)	p value
Male/female	10/8	9/12	0.037
Age (yr)	35 10	45 14	0.018
Fever	0	13	
Weight loss	0	7	
Arthralgia/arthritis	0	7	
Myalgia/myositis	0	5	
Facial pain	3	5	
Ear involvement	0	7	
Orbital pseudotumor	0*	2	
Subglottic stenosis	0	6	
Bronchial involvement	0	4	
Lung involvement	0†	12	
Skin involvement	0	4	
Multineuritis-cranial nerves	0	7	
RBC 1,000/mL	4,665 474	3,914 814	0.002
Hemoglobin (g/dL)	14.2 2.0	11.1 2.1	0.000
WBC /mL	10,305 2,868	10,690 4,872	0.776
Platelets 1,000/mL	333 69	440 193	0.037
CRP (mg/L)	12.2 12	79.4 73.7	0.002
ESR (mm/hr)	31 25	66 41	0.009
Total protein (g/dL)	7.6 0.6	6.6 0.6	0.000
Albumin (g/dL)	4.1 0.6	3.4 0.774	0.003
Alpha2 globulin (%)	11.2 2.7	12.9 3.7	0.064
Gammaglobulin	18.5 5.1	19.5 3.9	0.538
ANA positive	3/16	6/18	
RF positive	0/8	5/11	
Low complement	0/9	0/18	
Serum creatinine	0.9 0.18	1.01 0.4	0.302
Microhematuria	2/17‡	13/21§	
Proteinuria	2/17‡	13/21§	

During follow-up, 2 patients developed orbital pseudotumor with diplopia due to infection propagating from the nose that promptly responded to antibiotic treatment.

†During follow-up, 1 patient developed a *Staphylococcus aureus* lung abscess which resolved rapidly with antibiotic treatment.

‡Only trace in different patients.

§Ranging from trace to 3 in the same patients.

ranged from 13 to 108 months (median, 52 mo). Nineteen patients were evaluated at the time of first diagnosis, 2 at the time of their first relapse. Five WG patients had generalized disease with renal involvement, 3 had limited disease involving only the nasal and tracheal mucosa. The remaining 13 patients had lung involvement and other systemic symptoms but no kidney involvement. All patients had biopsy-proven WG or satisfied the Chapel Hill Consensus Conference definition (35) of the disease.

Clinical and laboratory evaluations

Physical examination included inspection of the face, oral cavity, and oropharynx; inspection of the nasal cavities and nasopharynx using 0 and 30 rigid telescopes, 4 mm in diameter, and the flexible fiberscope. Multiple biopsies and samples for bacterial and fungal cultures were taken under endoscopic guidance. To monitor the clinical course of the disease, digital images of the relevant endoscopic features were archived.

Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete and differential blood counts, liver and kidney function tests, urinalysis and microscopy, and serology for hepatitis virus B and C and human immunodefiency virus (HIV), were performed in every patient. In addition, antinuclear antibodies (ANA), rheumatoid factor (RF), and complement levels were determined in most patients.

ANCA determination

All sera from CIMDL and WG patients were tested for ANCA in 3 different laboratories. ANCA tests were first performed at the time of the patients' clinical evaluation in the laboratory of the Department of Clinical Immunology of Spedali Civili, University of Brescia, Italy. Sera were analyzed by indirect immunofluorescence microscopy (IIF) on ethanol-fixed blood donor neutrophils following the standard procedure delineated at the first ANCA workshop (77). Sera were further tested by enzyme-linked immunosorbent assays (ELISA) for the presence of antibodies reacting with PR3 (PR3-ANCA) and MPO (MPO-ANCA). PR3, purified as previously described (65), and MPO obtained from a commercial source (Calbiochem Biosciences, Inc. La Jolla, CA) served as antigens in the assays.

Aliquots of the sera were subsequently analyzed at the Mayo Clinic (Rochester, MN) by IIF and ELISA for MPO-ANCA following a standardized test algorithm (68). For some of the WG patients only convalescent sera were available for testing at Mayo (see Table 2). In addition, the sera were analyzed in the research laboratory of 1 of the authors (US) by IIF for PR3-ANCA and human leukocyte elastase (HLE)-ANCA using ethanol-fixed HMC-1 cells that expressed recombinant PR3 (rPR3) or HLE (rHLE) (56, 69). Furthermore, the sera were tested for PR3-ANCA by captureELISA using purified neutrophil PR3 (Athens Research, Athens, GA) as target antigen (71). IIF results obtained using neutrophils were expressed qualitatively as showing characteristic cytoplasmic staining (C-ANCA) or perinuclear staining (P-ANCA). IIF results using HMC-1 cells expressing rPR3 or rHLE were reported as positive when characteristic IIF staining was detected at a serum dilution of 1:4 or higher that was not detectable on sham-transfected HMC-1 (HMC-1/VEC) control cells (69). Solid-phase assay results were expressed in arbitrary units.

Imaging studies

Sixteen of the 18 CIMDL patients underwent cross-sectional imaging. A total of 24 examinations were evaluated: 7 computed tomography (CT) studies in 5 patients, and 17 magnetic resonance (MR) studies in 11 patients. Four patients had more than 1 imaging study (range, 2–5). Sixteen

of the 21 WG patients were evaluated by cross-sectional imaging: 2 by CT and 14 by MR.

The imaging studies were obtained shortly after the endoscopic examination in all patients (range, 2–25 d). Of the CIMDL patients, 4 patients had additional examinations (n 8) during the follow-up (range, 7–52 mo). All MR studies were performed at the Department of Radiology of the University of Brescia using superconductive equipment (Siemens Magnetom SP 1.5 T, Symphony 1.5 T). In all MR studies, spin echo or turbo spin echo T2 sequences were acquired along with enhanced T1 sequences. All CT examinations were performed without contrast enhancement. Two of 5 CT studies were performed at the Department of Radiology, the others at different hospitals. All imaging studies were reviewed by 1 of the authors (RM).

We analyzed the degree of septal destruction, that is, its area, obtained by multiplying the maximum diameters of the eroded septum, erosion of adjacent nasal structures (inferior, middle, superior turbinates), lateral nasal wall (medial antral wall, lamina papyracea) and floor of the nasal cavity (hard and soft palate). A score was obtained by assigning 1 point for each single structure involved. We evaluated abnormal changes of signal intensity of the mucosa on plain and enhanced MR sequences. In addition, we evaluated changes in size and signal of Waldeyer ring sites and abnormalities of the middle ear, and compared imaging studies obtained at the time of the first endoscopic examination. Follow-up studies were considered separately.

Histopathologic evaluation

A total of 44 mucosal biopsies from nasal cavities and paranasal sinuses were evaluated from the 18 CIMDL patients. Five patients had more than 1 biopsy (range, 2–16). Sections were stained with hematoxylin-eosin. Orcein staining was used to evaluate elastic fibers, and periodic-acid Schiff and Ziehl-Neelsen stains were used to identify fungi and mycobacteria, respectively. Polarizing filters were used to identify birefringent foreign material. Additional sections were immunostained for lymphoid pan-B-cell, CD20; pan-Tcell, CD3; Natural Killer (NK) cell, CD56 (Bio-SPA, Milan, Italy); and nonlymphoid, pan-macrophage, CD68 leukocyte-associated antigens. Stains for EBV-associated latent membrane protein 1 and RNA were performed using immunohistochemistry (antiLMP1) and in situ hybridization (EBER-1,2), respectively. Twentynine nasal biopsies were obtained from 21 patients with WG and evaluated in the same fashion. (Unless otherwise indicated, all antibodies used were purchased from Dako, Milan, Italy.)

Statistical analysis

Quantitative variable analysis was performed using the nonpaired Student t-test. The qualitative histopathologic variables between the 2 groups were compared using the Fisher exact test. The radiologic variables between the 2 groups were compared using the chi-square test and the Pearson correlation coefficient. For all comparisons, p values of 0.05 or less were considered significant.

Results

Clinical and laboratory findings

All patients with CIMDL sought an otolaryngologic consultation because of longstanding symptoms including nasal obstruction, epistaxis, and severe facial pain. The most common findings at the first visit were diffuse necrotizing ulcerative lesions, extensive crusting, and septal perforation. Destruction of the septum and inferior turbinates was invariably found in all patients. In more severe cases it extended to the middle and superior turbinates and the lateral wall of the nose. The latter was entirely absorbed in 3 patients. Hard and soft palate perforations were present at initial presentation in 2 patients and became evident during follow-up in 3 additional patients (Figure 1). The lesions gave rise to dysphagia and nasal reflux, and substantially affected the patients' quality of life. An example of progressive destruction of the midline structures is shown in (Figure 2).

None of the CIMDL patients complained of symptoms suggesting ear, orbit, or lung involvement at initial presentation. During the course of the disease, 2 patients with severe destruction developed acute orbital symptoms caused by propagating infections associated with pseudotumor, proptosis, and diplopia. One patient developed a *Staphylococcus aureus* lung abscess. All these lesions resolved rapidly with appropriate antibiotic therapy. At presentation, constitutional symptoms such as fever, malaise, weight loss, as well as arthralgia or myalgia were universally absent in this patient group, and none of the patients had any symptoms or laboratory findings that would indicate a systemic disease process.

The clinical presentation of patients with WG was different. The degree of nasal destruction was less severe than in patients with CIMDL, and signs of other organ involvement were present in most of the WG patients. All 21 patients presented with nasal crusting, but only 3 with a septal perforation. None of the WG patients had involvement of the turbinates, lateral nasal wall, or palate. A comparison of the affected sites is shown in Figure 3.

In patients with WG, nasal symptoms were usually only 1 of several complaints. Clinical evidence of involvement of sites other than nose and sinuses were detected in all patients. Systemic symptoms and alterations in the blood tests were absent in only 2 WG patients at the time of initial presentation. Both were women with subglottic stenosis as the only extranasal manifestation of the disease. All other patients had constitutional symptoms and arthralgia or myalgia. The lungs, ears, and cranial nerves were the other sites most frequently affected.

Laboratory abnormalities were significantly more frequent in WG than CIMDL patients. Among the 21 WG patients anemia of chronic disease was found in 16, elevation of white blood counts in 12, and of platelet counts in 10. Increased levels of CRP and ESR were detected in 15 and 14 of the WG patients, respectively. Of the 18 CIMDL patients, 2 had anemia, 5 had elevated white blood counts, and 2 had elevated

platelet counts. Abnormal CRP and ESR values were found in 9 and 8 CIMDL patients, respectively, but the mean values were significantly lower than those found in WG patients (see Table 1). Microhematuria and proteinuria were present in 13 of 21 WG patients, even in the presence of normal serum creatinine values. Several WG patients also had ANA (6 of 18 tested) or RF (5 of 11 tested). All CIMDL patients had normal liver and renal function test results and tested negative for hepatitis B antigen, hepatitis C, and HIV antibodies.

Fifteen of 17 CIMDL patients tested at the initial visit had positive nasal cultures for *S. aureus.* Thirteen of 21 WG patients were on broad-spectrum antibiotics at the time of diagnosis and, therefore, were not tested for *S. aureus.* Five of the remaining 7 WG patients not exposed to antibiotics had positive *S. aureus* nasal cultures.

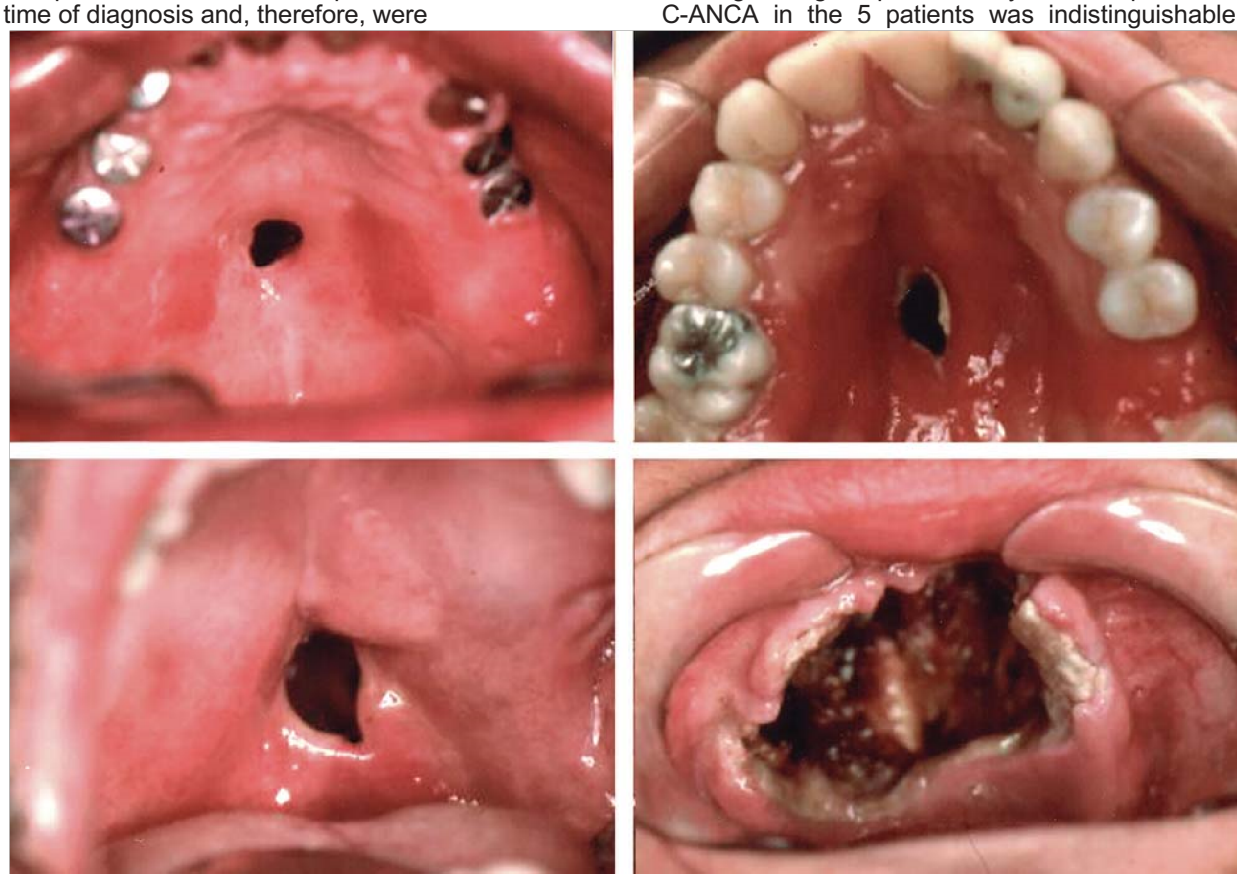

FIG. 1. Different examples of palate perforation in patients with cocaine-induced midline destructive lesions. The lesion can vary from a small defect to a large erosion involving the entire hard palate and extending to the alveolus.

ANCA results

All patients were tested for ANCA at initial presentation because WG was considered in the differential diagnosis of all necrotizing nasal lesions. A surprisingly large number of ANCA tests were positive in patients with CIMDL. The ANCA test results are summarized in Table 2. Only 5 of the 18 CIMDL patients were negative by all ANCA tests performed. In 8 patients, IIF was strongly positive for P-ANCA. All sera were negative for MPO-ANCA by ELISA. Three of the P-ANCA-positive sera reacted with rHLE, and 4 were positive in 1 or more of the tests performed to detect PR3-ANCA (direct ELISA, capture ELISA, or IIF on HMC-1/PR3 cells). Two of the P-ANCA-positive sera reacted with both HLE and PR3. Three of the PANCA-positive sera were negative for PR3-ANCA and HLE-ANCA.

Five patients with CIMDL had C-ANCA by IIF. All of them were positive for PR3-ANCA in at least 2 of the target antigen-specific assays. The IIF pattern of C-ANCA in the 5 patients was indistinguishable from that typically found in WG. IIF titers and direct ELISA assay units were as high as those encountered in WG patients. Two of the 5 C-ANCA-positive, PR3-ANCApositive sera also reacted with HLE. Two others were completely negative in the PR3-ANCA capture ELISA although they displayed strong reactivity in the other PR3-ANCA assays.

Of the 21 WG patients, only 2 were ANCA negative (see Table 2). These were the patients with biopsyproven disease affecting only the nose and subglottic area. Four patients had a P-ANCA pattern with corresponding positive MPO-ANCA

ELISA test results, and 14 had a C-ANCA pattern with matching PR3reactivity. Only 1 C-ANCA - positive WG patient reacted strongly with MPO but not PR3, a phenomenon that has been reported rarely (61). None of the ANCApositive sera from WG patients reacted with HLE.

Taken together, the data indicate that routine ANCA testing alone does not allow the differentiation between cocaine-induced and WG-associated nasal lesions. However, more detailed analyses of ANCA specificities suggest that the ANCA immune response in patients with CIMDL differs from that usually encountered in WG patients. In WG it is much more target-antigen restricted.

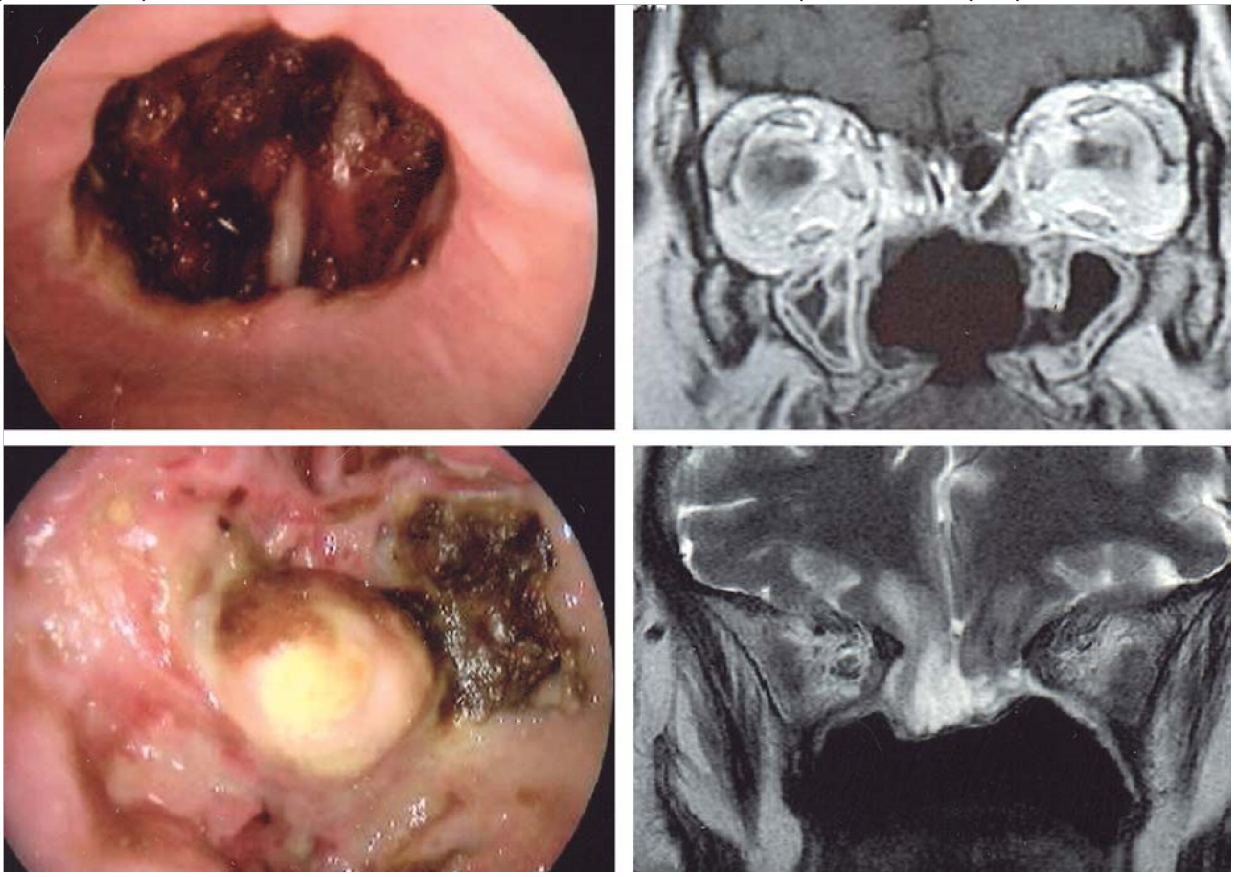

FIG. 2. Temporal progression of a midline destructive lesion in a patient with cocaine-induced midline destructive lesions over 3 years. In 1996, a large hard palate perforation was evident at inspection of the oral cavity (upper left panel). Magnetic resonance (MR) studies also demonstrated destruction of the inferior, middle, and superior turbinates on both sides and partial reabsorption of the left medial wall (upper right panel). By 1999, the lesion had progressed dramatically to destruction of the central part of the anterior skull base. The exposed dura and both frontal recesses are clearly visible in the endoscopic picture taken with a 70 scope (lower left panel). MR image shows the presence of a huge meningoencephalocele (lower right panel).

Radiographic findings

To identify imaging characteristics that help to differentiate cocaine-induced lesions from WGassociated lesions, we analyzed the cross-sectional imaging studies obtained at initial presentation in patients of both groups (Table 3). All CIMDL patients had septal perforations. Twelve of 16 (75%) had at least partial destruction of the inferior turbinate, which was bilateral in 9. Ten of the 16 cocaine abusers (62.5%) had partial or total destruction of at least 1 middle turbinate, which was bilateral in 5. Erosion of the superior turbinates was identified in 2 patients. The lateral nasal wall was eroded in 5 of the 16 (31.25%) patients. All had destruction of the medial maxillary wall; in 1 patient the erosion extended to the lamina papyracea. The floor of the nasal cavity was eroded in 4 of 16 patients (25%). In 1 of them the involvement extended to the soft palate.

In contrast, the imaging studies obtained at initial presentation in WG patients revealed a nasal septal perforation in only 2 of 16 (12.5%). The difference in frequency of nasal septal perforation was statistically significant (p 0.0005) with a positive predictive value of 88.9% for CIMDL. The involvement of a second nasal structure in addition to the nasal septum was discriminating between the 2 groups, as it was present in 75% of CIMDL patients but not in any of the WG patients.

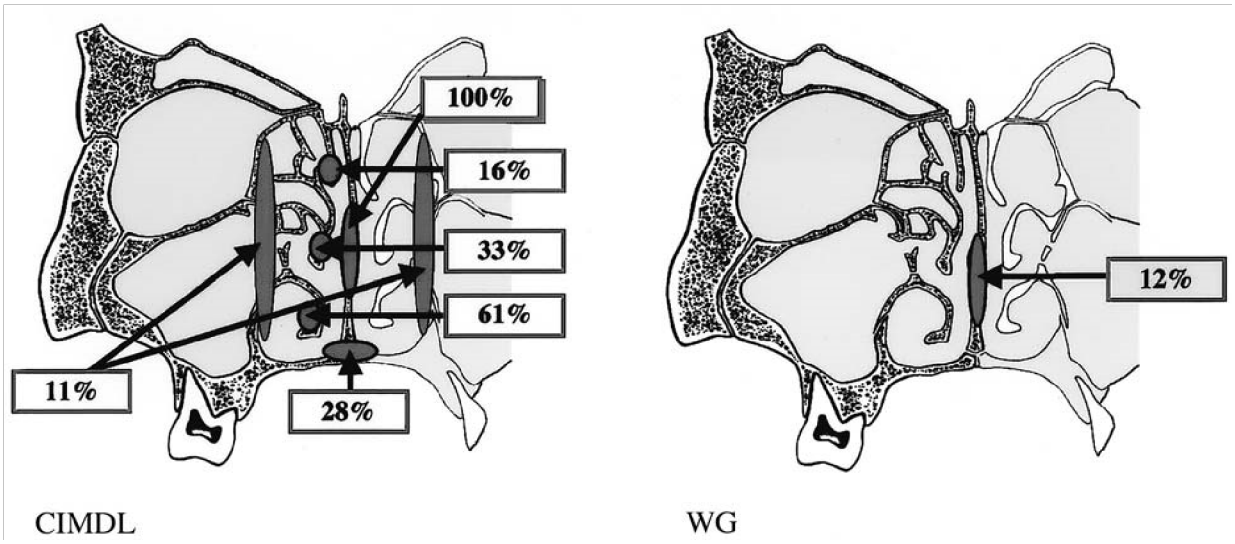

FIG. 3. Comparison of anatomic sites involved in cocaine-induced midline destructive lesions (CIMDL) and Wegener granulomatosis (WG) patients. The percentages indicate the proportion of patients of each group that had involvement of the indicated sites.

Accordingly, the average radiographic score of affected structures was significantly higher in CIMDL patients (4.06; range, 1–10), compared with WG patients (0.13; range, 0–1; p 0.001). A correlation was present between the extent of nasal septum perforation (mm^2) and the score. The correlation index was 0.803 in CIMDL patients (p 0.001) and 0.708 in WG patients (p 0.002). In addition, the occurrence of hard palate perforation in CIMDL patients was correlated with the destruction of bilateral inferior (Pearson 0.509; p 0.044) or bilateral middle (Pearson 0.545; p 0.029) turbinates.

In CIMDL patients, areas of an abnormal signal of nasal or paranasal mucosa such as hypointensity on T2 and reduced or nonhomogeneous enhancement were detected on 4 (36.36%) and 6 (54.54%) of the 11 MR scans, respectively. On the 14 MR scans obtained in WG patients, hypointensity on T2 and reduced or nonhomogeneous enhancement were present in 3 (21.4%) and 6 (42.8%) patients, respectively. Although MR abnormalities of the nasal mucosa were detected more frequently in CIMDL than in WG patients, the difference was not statistically significant. However, when only the mucosa of the central facial structures, that is, the nasal septum and the adjacent inferior and middle turbinates, was considered, reduced or nonhomogeneous enhancement was significantly more frequent in CIMDL than in WG patients (45.45% versus 16.66%; p 0.05). Abnormal thickening of the mucosa of the paranasal sinuses was observed more frequently in WG (78.57%) than in CIMDL patients (54.54%), but the difference was not significant.

Significant enlargement of the palatine or pharyngeal tonsils was found on the imaging studies in 9 of 16 (56.25%) CIMDL patients. It was associated with small fluid collections within lymphatic tissue in most cases. Similar chronic inflammatory changes of the Waldeyer ring were significantly less common in WG patients (n 2, 12.5%; p 0.05).

Radiographic signs of otitis media were detected in only 1 CIMDL patient but in 5 WG patients (31.25%). All had different degrees of hearing impairment. In 1 of the WG patients, enhanced tissue within the middle ear was detected which turned out to be granulomatous tissue causing destruction of the ossicle chain. The difference of radiographic middle ear abnormalities did not reach statistical significance.

Follow-up imaging studies were obtained in 4 of the CIMDL patients. All of them admitted to continued cocaine abuse. All showed progressive erosion of nasal structures. In 1 patient there was progressive “centrifugal” involvement of the lateral nasal walls and floor with eventual destruction of the entire framework and complete erosion of the hard and soft palate. The central floor of the anterior skull base was also eroded (see Figure 2).

TABLE 2. ANCA test results in 18 patients with CIMDL and 21 patients with nasal destructive lesions caused by WG*

Patient ID	ANCA-IIF (Brescia/Mayo)	MPO-ANCA ELISA (Brescia/Mayo)†	PR3-ANCA ELISA (Brescia)‡	PR3-ANCA Capture ELISA (Mayo)§	HMC-1/ PR3-IIF (Mayo)	HMC-1/ HLE-IIF (Mayo)
			CIMDL			
CIMDL 1	/	/				
CIMDL 2	/	/				
CIMDL 3	/	/				
CIMDL 4	/	/				
CIMDL 5	/	/				
CIMDL 6	P-ANCA	/				
CIMDL 7	P-ANCA	/				
CIMDL 8	P-ANCA	/				
CIMDL 9	P-ANCA	/				
CIMDL 10	P-ANCA	/				
CIMDL 11	P-ANCA	/	38.7			
CIMDL 12	P-ANCA	/		0.504		
CIMDL 13	P-ANCA	/	145	0.775		
CIMDL 14	C-ANCA	/	112	0.836		
CIMDL 15	C-ANCA	/	12.5	0.140		
CIMDL 16	C-ANCA	/	139	0.405		
CIMDL 17	C-ANCA	/	155			
CIMDL 18	C-ANCA	/	400			
			WG			
WG 1	/weak p-ANCA	/				
WG 2	/ND	/ND		ND	ND	ND
WB 3	p-ANCA/**	1000/**		**	**	**
WG 4	p-ANCA/**	84.7/**		**	**	**
WG 5	p-ANCA**	1000/**		**	**	**
WG 6	p-ANCA**	203/**		**	**	**
WG 7	c-ANCA	/	115			
WG 8	c-ANCA	/	***			
WG 9	c-ANCA	/	356	0.290		
WG 10	c-ANCA	/	285.9	0.490		
WG 11	c-ANCA	/	44.8	0.420		
WG 12	c-ANCA/**	/**	197.2	0.185**	**	**
WG 13	c-ANCA	/	10.1	0.417		
WG 14	c-ANCA	/	257	0.210		
WG 15	c-ANCA	/	132.5	0.340		
WG 16	c-ANCA	/	400	0.290		
WG 17	c-ANCA	/	***	0.660		
WG 18	c-ANCA	/	30.6	0.350		
WG 19	c-ANCA	/	108.5	0.300		
WG 20	c-ANCA/ND	/ND	69	ND	ND	ND
WG 21	c-ANCA[3]/ND	1000††/ND		ND	ND	ND

Abbreviations: ND not done.
* negative test result; positive.
†Negative: 10 U.
‡Negative: 15 U.
§Negative: 0.099.
**A subsequent convalescent serum was evaluated at Mayo.
* **The assay used was an anti-acidic PMN granule extract assay.
††Different sera from this patient were tested repeatedly, always generating the same result.

Comparison of the imaging abnormalities detected in CIMDL and WG patients corroborated the clinical findings indicating that the severity of tissue destruction is much more severe in CIMDL than in WG patients.

Histopathologic findings

To determine which histopathologic features are discriminating for CIMDL and WG, we reviewed all nasal biopsy specimens of each patient. Of the 44 biopsy specimens obtained from the CIMDL patients, 19 showed nonspecific changes consisting of fibrosis with mild inflammation or extensive necrosis. The remaining 25 biopsies (57%) showed significant histologic abnormalities (Table 4). In all these biopsies, a dense inflammatory infiltrate of mononuclear cells admixed with neutrophils and eosinophils was present. Nuclear atypia was universally absent. The inflammatory cells frequently encroached upon the wall of venules and arterioles, resulting in variable degrees of narrowing of the lumen. The feature, referred to as "perivenulitis," was found in 96% of the biopsies (Figure 4A). Microabscesses involving the wall of venules were found in 10 (40%) biopsies (Figure 4B). Obvious leukocytoclastic vasculitis with fibrinoid necrosis was identified in only 7 biopsies (28%) (Figure 4C). Other vascular changes detected in 7 (28%) biopsies consisted of fresh thrombi or sclerotic changes of the vascular wall, likely representing postthrombotic scars (Figure 4D). In 4 samples, the abnormalities involved medium-sized arterioles. It is noteworthy that the leukocytoclastic vasculitis and arteriolar thrombosis coexisted only in a single biopsy. Intra- or extravascular granulomas, scattered giant cells, or deep tissue necrosis with microabscesses were not found in any biopsy of the CIMDL patients.

TABLE 3. Radiographic changes

Site involved	CIMDL	WG	Yates	^{2}
Septum	16	2	p0.0000	p0.0000
Inferior turbinates	12	0	p0.0001	p0.0000
Inferior turbinates (bilateral)	9	0	p0.0017	p0.0004
Middle turbinates	10	0	p0.0006	p0.0001
Middle turbinates (bilateral)	5	0	p0.0515	p0.0149
Superior turbinates	2	0	p0.4652	p0.1441
Lateral nasal wall	5	0	p0.0515	p0.0149
Lamina papyracea	1	0	p1.000	p0.3096
Hard palate	4	0	p0.1088	p0.0325
Soft palate	1	0	p1.0000	p0.3096

When the histopathologic features of CIMDL patients with and without ANCA were compared, it appeared that occurrence of leukocytoclastic vasculitis was more frequent in the ANCA-positive than ANCAnegative patients (41.6% versus 20%, respectively). However, the difference did not reach statistical significance (p 0.6).

The immunohistochemical composition of the mononuclear inflammatory cells showed a prevalence of CD3 T cells, with a few CD56 NK cells, and variable numbers of CD68 macrophages. CD20 B lymphocytes were rare or totally absent (data not shown). All biopsies were negative for infectious organisms as well as for birefringent foreign material. Immunohistochemical and in situ hybridization studies aimed at the detection of Epstein-Barr virus antigen and RNA were also negative (data not shown).

The histopathologic changes observed in biopsies obtained from the WG patients are also summarized in Table 4. Significant histologic abnormalities were noticed in 22 biopsies (76%). A dense inflammatory cell infiltrate, with cell composition, distribution, and immunohistochemical characteristics similar to those found in the CIMDL group was detected. Perivenulitis was recognizable in all biopsies (100%). Other vascular changes consisted of microabscesses in the vascular wall (50%), leukocytoclastic vasculitis with fibrinoid necrosis (64%), and fresh or organized thrombi (23%). Pathognomonic histopathologic features (15, 22) such as extravascular multinucleated giant cells (Figure 4E) or granulomas and microabscesses with deeply located necrosis (Figure 4F), were detected in 41% and 86% of biopsies, respectively.

In summary, biopsies with nonspecific changes were more frequent in CIMDL (44%) than in WG patients (24%), but the difference was not statistically significant. Microabscesses in the vascular wall and perivenulitis were observed with similar frequencies in both groups. Leukocytoclastic vasculitis and fibrinoid necrosis appeared to be more frequent in WG (p 0.02). However, when the data analysis was based on the occurrence of the lesion in individual patients rather than individual biopsies, no difference was detectable: it occurred in 6 of 18 CIMDL and in 9 of 21 WG patients (p 0.11). In contrast, extravascular changes consisting of stromal granulomas with giant cells, microabscesses, and deeply located necrosis were features exclusively encountered in WG (p 0.001).

TABLE 4. Histopathologic features in nasal biopsies from 18 patients with CIMDL and 21 patients with WG

	CIMDL (no. biopsies/total)	WG (no. biopsies/total)	p Value (Fisher exact test)
Nonspecific changes	19/44	7/29	NS
Vascular changes			
Microabscesses in the vascular wall	10/25	11/22	NS

Lymphohistiocytic infiltrate ("perivenulitis")	24/25	22/22	NS
Thrombi (fresh or organized)	7*/25	5/22	NS
Granulomatous vasculitis	0/25	0/22	NS
Leucocytoclastic vasculitis and fibrinoid necrosis	7/25	14/22	0.02
Extravascular changes			
Single multinucleated giant cells or granulomas	0/25	9/22	0.001
Microscopic foci of deeply located necrosis, associated or not with microabscesses	0/25	19/22	0.001

*In 4 cases involving medium-sized arterioles.

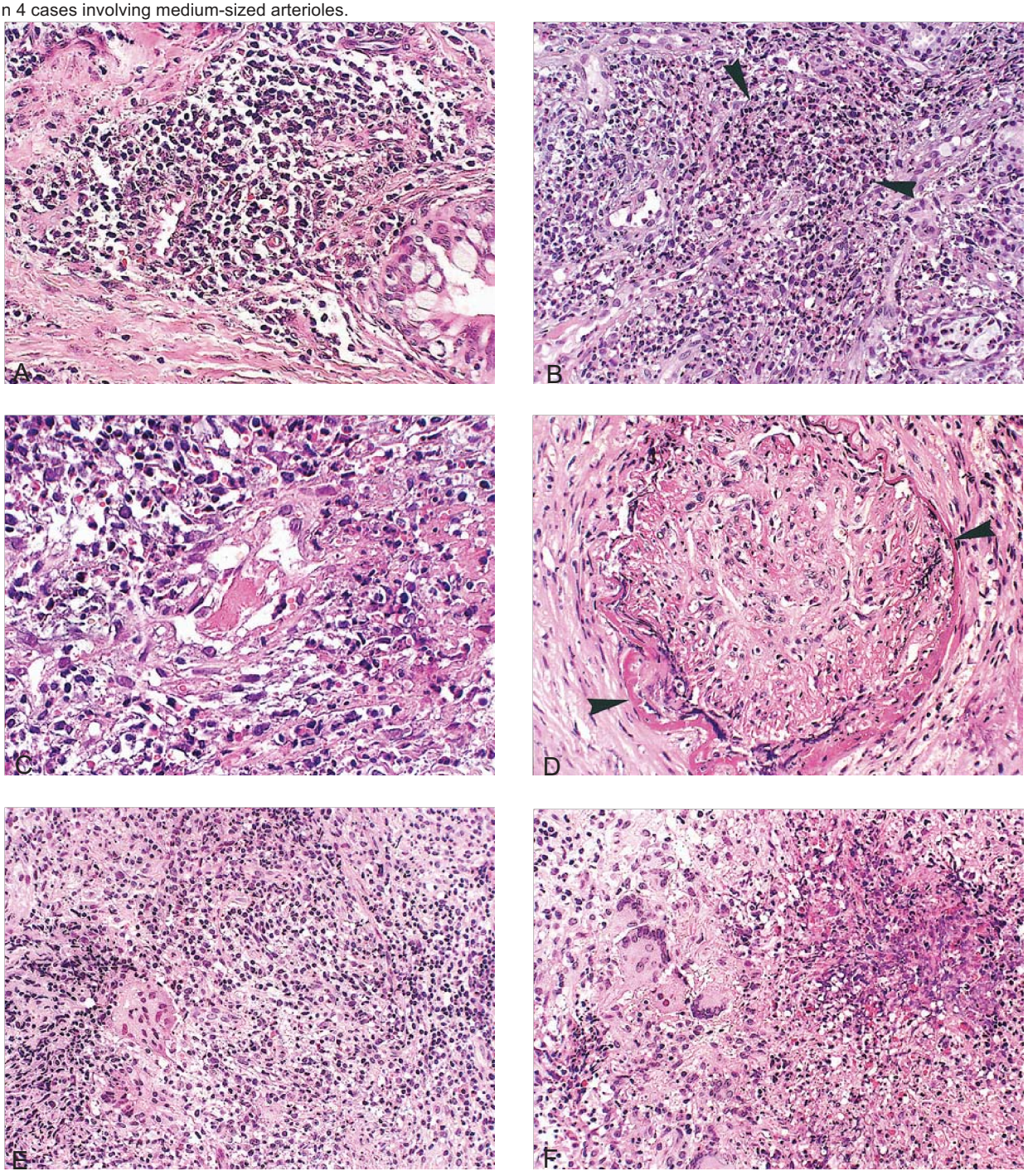

FIG. 4. Histopathologic features of nasal biopsies from cocaine-induced midline destructive lesions (panels A-D) and Wegener granulomatosis (WG) (panels E and F) patients. **A.** Dense polymorphic perivenular infiltrate, narrowing the lumen without obvious destruction of the vessel wall, referred to as "perivenulitis." **B.** The inflammatory infiltrate is particularly dense, and the formation of microabscesses within the vascular wall is recognizable (arrowheads). **C.** Fibrinoid necrosis and nuclear fragmentation identify leukocytoclastic vasculitis. **D.** The lumen of a small artery is occluded by scarlike tissue, probably resulting from an organized thrombus. Arrowheads indicate the external elastic lamina. **E** and **F.** Histopathologic features only seen in WG lesions are

multinucleated giant cells scattered within a dense inflammatory infiltrate (**E**) and associated with deep tissue necrosis (**F**). All biopsies were stained with hematoxylin and eosin. (Original magnification for A, 190 ; B, D, E, F, 95 ; C, 285).

Discussion

The abuse of cocaine may cause complications that can affect any organ system (16). Nasal septal perforation was first reported in 1912 (52). Despite the widespread abuse of cocaine, reports of CIMDL are scarce, and its incidence remains unclear. To our knowledge, only 24 cases with adequate clinical and pathologic documentation have been reported to date (4, 6, 8, 17, 20, 31, 38, 39, 43, 49, 58, 60, 62, 63, 66, 75). Only 5 of the reports (4, 17, 31, 62, 66) include ANCA test results, most of which were negative. The 2 reports of positive ANCA test results do not include target antigen specifications (4, 66). Based on its high specificity for the small vessel vasculitides including WG, a positive ANCA test result was thought to be of differential diagnostic value in this setting (62). In contrast, most of our patients presenting with CIMDL had ANCA. Because of this association they were at risk to be misdiagnosed as having WG. Indeed, some were initially treated with cytotoxic agents to no avail. Therefore, the identification of clinical, radiographic, and histopathologic features that allow a distinction between the 2 entities is important.

Clinical and radiographic differentiation of CIMDL and WG

Comparing the clinical and radiographic findings in patients with CIMDL with those of WG patients clearly indicates that the degree and localization of inflammatory changes differ substantially between the 2 groups. Furthermore, centrifugal progression of the necrosis from the nasal septum toward the nasal walls was apparent in CIMDL patients. In addition to the ubiquitous nasal septum perforation, most CIMDL patients had erosion of 1 (75%) or both (56.25%) inferior turbinates. Destruction of the middle turbinates (62.5%) occurred only in patients who also had inferior turbinate involvement. Among 18 cases reported in the literature with detailed imaging data (1, 2, 4, 8, 9, 17, 31, 38, 49, 60, 62, 66, 74, 75), 1 or both inferior turbinates were affected in all patients, and 1 or both middle turbinates were involved in 89%. The difference may reflect referral and reporting bias. Further destruction of the midfacial architecture involving the lateral nasal wall (medial maxillary sinus wall 31.25%, lamina papyracea 6.25%) or nasal floor structures (hard palate 25%, soft palate 6.25%) was always associated with involvement of inferior and middle turbinates. The notion of centrifugal progression is corroborated by the strong correlation between the extent of septal perforation and the number of nasal structures eroded. In contrast, erosion of structures of the midline was rare among WG patients (12.5%) and was limited to the nasal septum.

The localization of mucosal inflammation detected on imaging studies may provide further clues about the etiology. Hypointensity of mucosa and submucosal tissues on T2 and reduced or nonhomogeneous enhancement have recently been suggested as markers for granulomas and vasculitis in WG (47). In our study, these 2 abnormal MR signal patterns were not only detected in WG but also among cocaine abusers with similar frequency. In both patient groups, nonspecific inflammatory mucosal changes of the paranasal sinuses were identified. However, in CIMDL patients, signal intensity abnormalities were concentrated in the nasal septum and the turbinates. The central structures probably represent the areas of drug deposition. In contrast, the lesions had a more scattered distribution in WG, thereby reflecting more diffuse inflammation. Finally, abnormal enlargement and cystic changes of lymphatic tissue of the Waldeyer ring suggesting chronic reactive inflammatory changes were significantly more frequent in cocaine abusers.

Laboratory testing aimed at the detection of chronic or systemic inflammation, liver and kidney function abnormalities, infectious agents, and markers of autoimmunity revealed no significant abnormalities in CIMDL patients, representing a stark contrast between the severe destruction of the nose and an otherwise good health status.

It cannot be excluded that the observed clinical and radiographic differences between CIMDL and WG reflect referral bias. It is indeed possible that patients with cocaine-induced lesions seek medical attention only after substantial tissue damage has occurred. In contrast, WG patients may be identified earlier for several reasons including symptoms in other organ systems. However, when severe destruction of nasal structures, bony erosion, and signs of inflammation affecting the lymphoid tissue of the Waldeyer ring are encountered in the absence of systemic inflammatory changes, substance abuse should be suspected and carefully ruled out.

Histopathologic differentiation of CIMDL and WG

Histopathology remains the “gold standard” of diagnosis for WG, particularly in patients with disease limited to the upper respiratory tract. Biopsies of the nose and sinuses have a limited sensitivity which hinges on the identification of pathognomonic histopathologic features of WG in the specimen. Vascular changes including microabscesses in the wall of venules and leukocytoclastic vasculitis have been proposed as characteristic features of WG that should be sought in head and neck biopsy specimens (15, 19, 22). We found vascular changes including chronic perivenulitis, microabscesses, and frank leukocytoclastic vasculitis in the majority of CIMDL patients (57%). In addition, thrombosis of venules and arterioles was found in the form of fibrin deposition or organizing intravascular granulation tissue. Consequently, the histologic changes observed in a large proportion of biopsies from patients with CIMDL might be misinterpreted as “consistent with WG” (15). Previous case studies of CIMDL reported mucosal biopsies showing nonspecific tissue necrosis and acute or chronic inflammation in the absence of vasculitis or granulomas (4, 31, 38, 49, 62, 66). This discrepancy is difficult to interpret because the timing and the size of the biopsies were not reported. It is possible that at our institution biopsies were performed early in the disease process and that the specimens were larger. The occurrence of vasculitis in nasal

biopsies from cocaine abusers is not entirely unexpected, since cocaine-associated vasculitis has been reported to occur in other sites, such as the central nervous system and the skin (23, 25, 37, 44, 46, 51), and occlusive arteriolar lesions have been found in the gastrointestinal tract (33). Furthermore, in vitro experiments have documented increased adhesion of neutrophils and monocytes to endothelial cells, increased expression of endothelial adhesion molecules ICAM-1, VCAM-1, and ELAM-1, and increased expression of TNF- and interleukin-6 (26), mechanisms that have also been implicated in ANCAassociated vasculitis.

Even though leukocytoclastic vasculitis appeared more frequently in nasal biopsies of WG than CIMDL patients, the finding does not exclude CIMDL. Furthermore, others have detected leukocytoclastic vasculitis in a much lower percentage of nasal biopsies from WG cases (22). Therefore, this histopathologic feature alone is of low sensitivity and specificity for WG.

Perivenulitis is characterized by a dense polymorphic infiltrate that surrounds the vessels and encroaches upon the vascular wall and narrows the lumen. It is not associated with leukocytoclasia or fibrinoid necrosis, but it can mimic genuine vasculitis. Similar vascular abnormalities have been reported in WG, with different interpretations ranging from chronic inflammation of the vessel walls to nonspecific changes (21, 22, 73). We found the feature in almost all biopsies from both groups and, therefore, believe it is nonspecific. Granulomatous vasculitis was not detected in any of the biopsy specimens of WG patients, in accord with the results of others (19, 22).

In contrast to the vascular changes, the nature of the extravascular inflammatory changes is more discriminating. Scattered multinucleated giant cells, granulomas, and extravascular foci of microscopic necrosis were identified in a large proportion of WG cases but not in any of the biopsies obtained from cocaine abusers. In conclusion, a definitive differential diagnosis between CIMDL and WG cannot be made on the basis of vascular changes on mucosal biopsies from the upper respiratory tract. However, it is possible, provided the specific extravascular changes of WG are found. This observation highlights the importance of extravascular changes in the histologic diagnosis of WG (14, 15, 24, 41). It also reemphasizes that WG is a necrotizing granulomatosis and a vasculitis, a view that was held by Wegener himself and others (15, 24, 41, 42, 64, 76).

ANCA in CIMDL

The high frequency of ANCA in patients with CIMDL was unexpected and raises questions about their etiology, their pathogenic significance, and whether they can be distinguished from the ANCA in WG. The positive ANCA tests in patients with CIMDL may lead to a clinical misdiagnosis, particularly if only IIF testing or antigen-specific ELISAs are performed in isolation. Several recent multicenter studies and consensus statements have called for the combined use of IIF and target antigen-specific testing, because only this combination maximizes the sensitivity and specificity of ANCA testing for vasculitis (30, 59). Indeed, very few well-documented occurrences of C-ANCA with matching PR3-reactivity and P-ANCA with matching MPO-reactivity have been reported in nonvasculitic conditions (10, 45, 53). Our data seem to confirm this only partially. None of the P-ANCA found in CIMDL patients reacted with MPO, whereas all P-ANCA found in the WG control population reacted as expected with MPO. Further testing revealed that 4 of the P-ANCA found in CIMDL patients reacted with PR3, a phenomenon which has been reported to occur occasionally, but not in patients with well-documented biopsy-proven WG (36, 69). Three of the P-ANCA positive sera reacted with HLE, and 2 of them were positive for both PR3 and HLE, suggesting crossreactivity or occurrence of different ANCA in the same specimen. Consequently, target antigen-specific analysis of the P-ANCA-positive sera reveals a clearcut difference from the P-ANCA seen in WG or microscopic polyangiitis.

The 5 C-ANCA-positive sera found in CIMDL patients represent more of a diagnostic challenge because they all reacted with PR3 in at least 2 target antigen-specific assays. More detailed testing revealed that 2 of them also reacted with HLE. The frequent occurrence of HLE-ANCA (5 of 13) may distinguish the ANCA of CIMDL from those of vasculitis. HLE-ANCA have been described in autoimmune diseases, but are extremely rare in vasculitis (12, 40, 48). Only 1 study using an ELISA for their detection reported 8 patients with HLE-ANCA among 108 WG patients and 15 among 78 microscopic polyangiitis patients (3). In the WG population presented here, no HLE-ANCA reactivity was found. Using the same HLE-ANCA detection method applied here, we tested a cohort of 615 consecutive patients being evaluated for possible vasculitis and identified no HLE-ANCA in that patient population (56).

Finally, the fact that 2 of the 5 C-ANCA-positive sera (40%) were negative in the capture ELISA further sets these ANCA apart from those typically found in WG. These PR3-ANCA compete for the epitope on PR3 that is recognized by the capturing monoclonal antibody, MCPR3–2, an epitope that is recognized by less than 5% of PR3-ANCA from WG patients (57, 71).

The cause of ANCA in CIMDL patients remains unclear. In this context the observation that 4 of the 13 ANCA-positive CIMDL patients but none of the 19 ANCA-positive WG patients displayed doublereactivity is instructive. ANCA reacting with multiple target

antigens at the same time have previously been noted in patients with drug-induced ANCA and vasculitis (reviewed in references 11, 34). Perhaps the ANCA response in CIMDL patients is the result of polyclonal B-cell stimulation by cocaine or drug adulterants similar to that induced by propylthiouracil, hydralazine, D-penicillamine, allopurinol, and others.

It is also possible that ANCA in CIMDL patients are related to infection with *S. aureus,* which was documented in most of the patients. This is consistent with reports linking the almost universal presence of nasal *S. aureus* in cocaine abusers to a high risk of infectious complications including toxic shock syndrome after nasal surgery (27). The mucosal damage caused by ischemia or by crystal-induced microtrauma may predispose cocaine users to the development of chronic low-grade infection with *S. aureus*. WG patients' nasal mucosa is also frequently chronically infected with *S. aureus,* and the carrier status has been linked to a higher relapse rate as well as to ANCA formation (70). Several mechanisms have been proposed to explain the role of *S. aureus* infection in ANCA formation (reviewed in reference 13). *S. aureus* releases toxins known to be powerful superantigens thought to activate T cells and B cells in an unrestricted manner, by circumventing the normal antigen-specific immune response. Furthermore, cell-wall components of *S. aureus* are known to be effective T-cell-independent B-cell mitogens that could induce autoreactive B cells to produce ANCA.

Many studies have reported ANCA in various infections (reviewed in references 10, 34). For most, the target antigens for the ANCA were not PR3 or MPO (10). However, C-ANCA reacting with PR3 have been well documented in subacute bacterial endocarditis (10) and in shunt nephritis (7). Streptococcal species were identified in 5 of the 7 reported endocarditis patients (10). Streptococcal species are also known to produce superantigens (18, 54). All patients recovered on antibiotic therapy, and ANCA disappeared after resolution of the infection (7, 10). In contrast, chronic infections of the necrotic CIMDL are almost impossible to eradicate. This may contribute to the persistence of ANCA in these patients. PR3- and MPO-ANCA have been implicated in the pathogenesis of small vessel vasculitis (reviewed in references 32, 34, 50). At first, our finding of ANCA in CIMDL patients seems to speak against a pathogenic role of ANCA because none of the CIMDL patients developed any sign of vasculitis. Furthermore, the clinical presentation and histopathologic features did not differ significantly between CIMDL patients with ANCA and those without. However, there is growing evidence that different ANCA subsets may have different pathogenic potential. Many large studies performed during the last decade indicate that only ANCA reacting with the target antigens PR3 or MPO, and not those directed against other target antigens, are related to the development of vasculitis (reviewed in reference 34). In addition, PR3-ANCA and MPOANCA from vasculitis patients appear to recognize a restricted number of conformational epitopes (5, 67, 72). Some conformational PR3-ANCA epitopes, such as those displayed preferentially on the proform of PR3, seem to correlate more closely with vasculitis activity than others (55). Our more detailed characterization of the ANCA detected in CIMDL patients indicates both reactivity with target antigens other than those associated with vasculitis, and evidence that the PR3-ANCA found recognize a different spectrum of epitopes than the typical PR3-ANCA in WG.

Conclusion

We found a high frequency of positive ANCA test results in patients who presented for an evaluation of severe necrotizing nasal lesions associated with habitual nasal cocaine insufflation. As the drug use history provided by patients is notoriously unreliable, this finding may complicate the differentiation of cocaine-induced lesions from necrotizing granulomatous inflammation of the upper respiratory tract associated with WG. Careful physical, radiographic, and histopathologic examination of the CIMDL patients and all WG patients seen with nasal inflammation during the same time frame revealed significant differences between the groups. The localized destruction of nasal and facial structures is much more severe in CIMDL, whereas signs of systemic inflammation are universally absent. Vascular abnormalities mimicking vasculitis were frequently found in biopsy specimens of CIMDL patients and are not helpful in the differential diagnosis. However, extravascular necrosis, microabscesses, granulomas, and giant cells are differentiating histopathologic hallmarks of WG. Whereas routine ANCA testing does not clearly differentiate the ANCA found in some CIMDL patients from those of WG patients, more detailed investigations suggest interesting differences between the ANCA of the 2 patient populations.

Summary

We compared the clinical, serologic, radiographic, and histopathologic features of 18 consecutive patients who presented with cocaine-induced midline destructive lesions (CIMDL) with those of all 21 patients with Wegener granulomatosis (WG) with nasal involvement evaluated during the same time period. Routine ANCA tests were positive in 13 of 18 CIMDL patients compared with 19 of 21 WG patients. Clinical and radiographic evaluation revealed that destruction of facial midline structures was significantly more severe in CIMDL than WG. In contrast to WG, there was no other organ involvement and no significant laboratory

abnormalities indicating systemic inflammation in CIMDL. Histopathologic evaluation revealed the frequent occurrence of nonspecific inflammation, necrosis, and vascular abnormalities such as leukocytoclastic vasculitis and perivenulitis in CIMDL as well as in WG. Only extravascular microabscesses, necrotizing granulomas, and multinucleated giant cells found in WG were discriminatory features. More detailed analysis of the ANCA found in CIMDL and WG patients showed the following differences. Of 8 PANCA-positive CIMDL sera, none reacted with MPO, 4 reacted with PR3, 3 reacted with HLE, 2 of which showed double-reactivity with PR3 and HLE. All of 5 C-ANCA-positive CIMDL patients showed reactivity with PR3. Two of these also reacted with HLE. In contrast, all but 1 of the 19 ANCA-positive WG patients displayed concurrent P-/MPO-ANCA or C-/PR3-ANCA reactivity, respectively. In 1 WG patient the target antigen reactivity was reversed. None of the WG patients displayed double-reactivity. Consequently, routine ANCA testing does not allow an unequivocal distinction between CIMDL and nasal involvement of WG, but more detailed investigations suggest instructive differences between the ANCA immune responses of the 2 patient populations.

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Massive apoptosis erodes nasal mucosa of cocaine abusers

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ABSTRACT

Background: *A threatening occurrence in some cocaine abusers is the progressive destruction of nasal structures (cocaine-induced midline destructive lesions [CIMDL]) that may end in a highly severe disease.*

Methods: *Thirty patients with CIMDL, 10 healthy patients, 10 patients affected by nasal polyposis, and 10 patients affected by Wegener granulomatosis were observed. Biopsy specimens of nasal mucosa were analyzed by immunohistochemistry for caspases-3, -9 and -8 and by the terminal deoxynucleotidyl transferase-mediated dUTP-digoxygenin nick end labeling (TUNEL) method. The time and concentrationdependent effects of cocaine in vitro were studied in HaCat cells by TUNEL and Western blotting.*

Results: *All CIMDL biopsy specimens showed abundant caspase-3 and caspase-9 expression but no caspase-8 positive cells. No obvious expression of any caspases was detected in biopsy specimens from healthy subjects or in patients affected by nasal polyposis or Wegener granulomatosis. In HaCat cells cellular changes were observed, which confirmed induction of massive apoptotic events. The rate of apoptosis in HaCat cells was dependent on the concentration of cocaine. After 1 hour, 2.5, 5, and 10 mM of cocaine induced 16, 45, and 84% of apoptotic figures, respectively, while 6 hours of exposure increased apoptosis to 25, 54, and 94% at the same concentrations. Caspase expression and activation in HaCat cells treated with 100 M and 1 mM of cocaine for 1 hour were confirmed by Western blotting.*

Conclusion: *Cultured epithelial cells show both time- and dose-dependent increases in apoptosis and cellular damage on cocaine treatment. We suggest that some abusers trigger CIMDL by abnormally boosting apoptosis within nasal epithelial cells. Cocaine abusers with higher apoptotic rates may predict whether they will eventually develop CIMDL.* (Am J Rhinol 20:160–164, 2006)

The National Household 1998 Survey on Drug Abuse re- sions, 10 healthy subjects, 10 patients affected by nasal polported that cocaine was used by 3.8 million Americans yposis (NP), and 10 patients affect by Wegener granulomatorepresenting 1.7% of the household population over 12 years sis (WG). Specimens were collected and examined since of age. Current estimates suggest that there are about 1.75 January 1991, at the Department of Otorhinolaryngology of million habitual cocaine abusers in the United States.[1] the University of Brescia, Italy (*n* 39) and at the Department

Habitual insufflation of cocaine powder often causes of Otorhinolaryngology, San Raffaele Hospital, Milan, Italy marked damage of the nasal mucosa and underlying peri- (*n* 21). chondrium that may infrequently lead to nasal septum per- Patients with CIMDL (18 men and 12 women) ranged in foration and destruction of the osteocartilagineous scaffold of age from 22 to 66 years (median, 37 years). At the time of first the nose, sinuses, and palate[2–4] (Fig. 1, *a*–*d*). Such individuals observation, all patients except three admitted cocaine use. In are affected by cocaine-induced midline destructive lesions nine patients reliable information on the duration of abuse (CIMDL) and display a clinical pattern mimicking systemic and doses could not be obtained. Four patients had a history diseases with positive anti–neutrophil cytoplasmic antibodies of abuse with an undetermined dose lasting 6, 7, 10, and 12 (ANCA) tests, radiographic abnormalities chiefly in the nasal years, respectively. One patient had been using 1–3 g/week septum and turbinates or in other sinonasal sites, as well as on an irregular basis. The remaining 13 patients had been marked histopathological changes.[5] In this report, we studied using cocaine for 2–30 years, at doses ranging from 1 to 20 the local effects of cocaine on samples of nasal mucosa of g/week. The 10 consecutive healthy subjects (7 men and 3 abusers and several control populations. The effects of cocaine women, aged 18–52 years; median, 32 years) underwent a *in vitro* also were studied in a human immortalized nontransformed epithelial cell line (HaCat) by the terminal deoxynucleotidyl transferase-mediated dUTP-digoxygenin (TUNEL)[6] assay and Western blotting to investigate the apoptotic and cytotoxic effects induced by cocaine.

METHODS

We reviewed samples of nasal septal biopsy specimens from 30 CIMDL patients with variable severity of nasal le- nasal biopsy during septoplasty. The other two control groups, 10 NP (7 men and 3 women; aged 18–69 years; median, 49 years) and 10 WG (4 men and 6 women; aged 30–64 years; median, 45 years), had anterior nasal septum biopsies. The effects of cocaine (Unipharma SA, Berbengo, Switzerland) in HaCat cells were evaluated by examining the extent of apoptotic pathways that were activated *in vitro* and *in vivo* with the aim of detecting a direct proapoptotic effect that was independent of tissue-derived factors.

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Cell Culture

HaCat cells were grown in Dulbecco's modified Eagle's medium (Gibco, Milan, Italy) supplemented with 10% fetal bovine serum (Gibco), 1% penicillin-streptomycin, and glutamine solution (Gibco) in 5% CO_2 at 37°C. Cells were seeded at a density of 10^4 cells/mL into 24-well plates and treated with different concentrations of cocaine (from 100 M to 2.5, 5, and 10 mM). In one set of experiments, cells exposed to 100

palate perforation with regular margins (*), tongue (k). (d) Nasal endoscopy (0° rigid fiberscope): typical nasal crusting in a cocaine abuser (left [°] and right [°°] inferior turbinates, 1.5-cm hard palate perforation [*] and residual nasal septum [§]).

M of cocaine also were treated with 20 M of caspase-3 inhibitor (Ac-DEVD-CHO; Calbiochem, Merck, Milan, Italy); Ac-DEVD-CHO was omitted. Samples were analyzed by light microscopy to quantify apoptosis. One hundred nuclei from random microscopic fields were anaand treated with 100 M and 1 mM of cocaine for 1 and 6 lyzed and the percentage of apoptotic cells were calculated as hours, respectively. Cells exposed to 1 M of staurosporine the number of apoptotic cell/total number of cells 100%. (Sigma, Milan, Italy) or 50 M of etoposide (Sigma) were used Each experiment was conducted in quadruplicate and reas positive controls. peated at least three times.

Tissue Samples of Nasal Mucosa and Immunohistochemistry

Biopsy specimens of nasal mucosa were removed surgically, fixed in 10% formaldehyde in 0.1 M of phosphate buffer at pH 7.4, and embedded in paraffin. Three-micron sections of nasal pyramid. (c) Oral endoscopy (0° rigid fiberscope): a 1.5-cm soft ride (BioGENEX, San Ramon, CA), counterstained with hematoxylin (Sigma), and mounted in Mountquick aqueous medium (Bio-Optica, Milan, Italy). Stained slides were observed in a Zeiss Axiophot microscope and pictures were acquired digitally.

In Situ TUNEL Assay

phosphate (Roche) in Tris-buffered saline (1 M of Tris-HCl and 5 M of NaCl), pH 9.5, in the dark for 10 minutes. Positive controls were incubated with 1 U of DNase/100 L mixture for 10 minutes at room temperature. Finally, stained sides were observed in a light microscope.

Quantitative Analysis of Apoptotic Cells

in controls cultures were analyzed by immunofluorescence and TUNEL analysis. For Western blotting, cells were cultured at a density of 10^6 hundred nuclei from random microscopic fields were anaand treated with 100 M and 1 mM of cocaine for 1 and 6 lyzed and the percentage of apoptotic cells were calculated as hours, respectively. Cells exposed to 1 M of staurosporine the number of apoptotic cell/total number of cells 100%. (Sigma, Milan, Italy) or 50 M of etoposide (Sigma) were used Each experiment was conducted in quadruplicate and reas positive controls. peated at least three times.

Protein Extraction and Western Blotting

After treatment, HaCat were pelleted and resuspended in radioimmunoprecipitation assay lysis buffer (10 mM of Triscally, fixed in 10% formaldehyde in 0.1 M of phosphate buffer HCl, pH 7.2, 1% deoxycholic acid, 1% Triton X-100, 0.1% at pH 7.4, and embedded in paraffin. Three-micron sections sodium dodecyl sulfate [SDS], 150 mM of NaCl, and 1 mM of

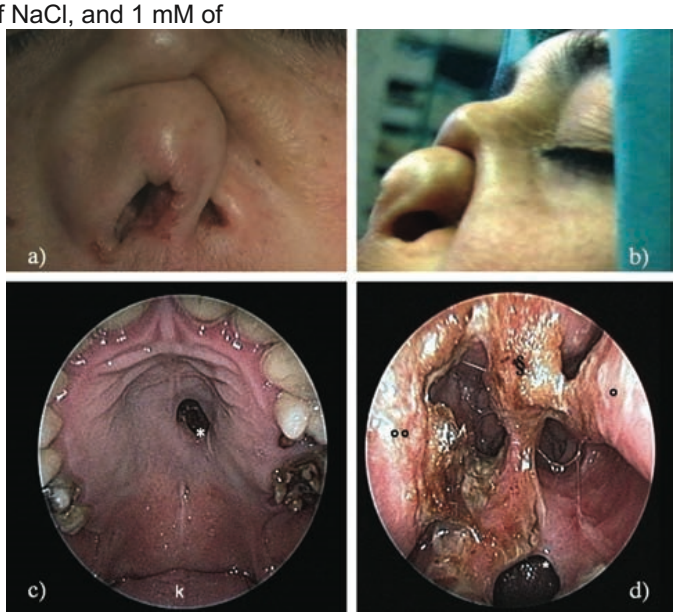

Figure 1. *Some examples of CIMDL lesions. (a and b) Columella and left alar cartilage collapse and severe destruction*

Apoptotic cells also were identified on tissue sections and HaCat cells, using a commercially available *In Situ* Cell Death Detection kit, AP (Roche, Monza, Italy) according to the manufacturer's instructions. Bioptic sections were hydrated in PBS and incubated in 0.1% Triton X-100 in 0.1% sodium citrate, and HaCat cells were fixed with freshly prepared 4% paraformaldehyde/3% sucrose and permeabilized in HEPES-Triton X-100 buffer. In both cases samples were exposed to 3% hydrogen peroxide solution to quench endogenous peroxidase. Apoptotic cells were labeled with the TUNEL reaction mixture. They were then conjugated with alkaline phosphatase by incubation in the dark with 50 L of converter-alkaline phosphatase for 30 minutes at 37°C in a water-saturated atmosphere. After substrate reaction, samples were treated with 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-

were used for immunohistochemistry with the Vectastain Elite ABC kit (Vector, Milan, Italy). Briefly, sections were hydrated in graded alcohol solutions and finally washed in distilled water, immersed in citrate buffer (0.1 M of citric acid/0.1 M of sodium citrate), boiled in a microwave oven three times for 5 minutes, and finally washed in phosphatebuffered saline (PBS). Slides were soaked in 3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity, blocked in normal serum for 1 hour at room temperature, and incubated for 30 minutes at 37°C with three different antibodies: monoclonal anti-human caspase-3 (Alexis, Rome, Italy), anti-human caspase-8 (Cell Signaling, Milan, Italy), and polyclonal anti-human caspase-9 (Alexis). Then, sections were incubated with a biotinylated anti-mouse immunoglobulin G (IgG) or anti-rabbit IgG secondary antibody for 1 hour at room temperature, followed by a 30-minute incubation with a preformed avidin and biotinylated peroxidase complex. They were then developed with diaminobenzidine tetrahydrochlo-

ethylenediamino-tetra-acetic acid [EDTA], pH 8) supplemented with 1 mM of phenyl-methylsulfonylfluoride (Sigma) plus the Protease Inhibitors Cocktail (Sigma, Milan, Italy), and incubated for 10 minutes at 4°C. After centrifugation at 14,000 rpm for 10 minutes, proteins were recovered and stored at 20°C. Protein concentration was determined by using the BCA Protein Assay Reagent Kit (Pierce, Celbio, Pero, Italy). Forty micrograms of proteins were separated on 12% SDSpolyacrylamide gels and blotted onto Immobilon-P Membranes (Millipore, Milan, Italy). The membranes were then blocked with 10% nonfat dried milk for 30 minutes at 37°C and washed three times in washing buffer (PBS containing 0.05% Tween-20). Membranes were incubated with primary antibody (caspase-3, -9, or -8) for 1 hour at room temperature, kept in washing buffer, and treated with specific secondary antibodies for 20 minutes at room temperature on a shaker platform. A sensitive enhanced chemiluminescent substrate (Supersignal West Dura Extended Duration Substrate; Pierce)

was used for detection and blots were exposed to Hyperfilm (Amersham Biosciences) for a few seconds.

RESULTS

All samplesfrom cocaine abusers showed abundant caspase-3 (Fig. 2, *a* and *e*) and caspase-9 expression (Fig. 2, *b* and *f*) but no caspase-8 cells (Fig. 2, *c* and *g*). Such cells were abundant within the superficial layer of the nasal epithelium and in the submucosa underneath the erosion area. No obvious caspase expression in the epithelium was detected in biopsy specimens taken from patients from unrelated pathologies. In the WG group, seven patients showed occasional and mild expression of caspase-9 within the cell population inhabiting the submucosa but no apoptotic figures were observed. The TUNEL assay approximately confirmed these results, suggesting that the density of apoptotic cells was a highly characteristic feature of the CIMDL abuser group (Fig. 2*d*) but was irrelevant in all the control groups (Fig. 2*h*).

Hoechst chromatin staining indicated segmented nuclei and apoptotic bodies in the same cells and counts confirmed massive time-dependent apoptosis on cocaine treatment that was totally prevented by the caspase-3 inhibitor Ac-DEVDCHO (Fig. 3*a*).

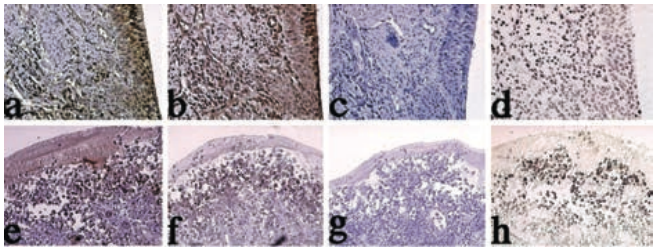

Figure 2. *Sections of nasal mucosa biopsy specimens obtained from cocaine abusers. The expression of (a and e) caspase-3 and (b and f) caspase-9 was detected with an immunohistochemistry assay. Apoptotic cells were detected not only on the superficial layer of epithelium, but also in the connective tissue underneath the area of erosion. (c and g) The expression of caspase-8 was not detected in all sections. (d and h) Apoptotic cells also were detected using TUNEL assay. TUNEL dark cells are present both in the epithelium and in the lamina propria.*

DNA Agarose Gel Electrophoresis

The characteristic pattern of oligonucleosome-sized fragments producing a DNA ladder on agarose gels is the biochemical hallmark of apoptosis. After treatment, cells were harvested and lysed in 20 mM of EDTA, pH 8, 50 mM of Tris-HCl, pH 7.5, 1% NP-40 (Sigma), and 1% of SDS containing 200 g of proteinase K (Sigma) at 55°C for 30 minutes. RNA was removed by the addition of 100 g of RNase A (Roche) at 37°C for 1 hour. DNA was extracted from the supernatant with the same volume of phenol-chloroformisoamyl alcohol (25:24:1), centrifuged at 7000 rpm for 15 minutes and precipitated with 0.1 M of sodium acetate and ethanol. The pellet was washed twice with 70% ethanol and dissolved in H_2O. Ten micrograms of DNA was electrophoresed at 70 V in a 1.8% agarose gel in Tris-acetate-EDTA buffer and stained with ethidium bromide. A 1 kb of DNA molecular weight marker was used for the analysis of internucleosomal DNA fragmentation.

The rate of apoptosis in HaCat cells depended on the concentration of cocaine: after 1 hour, 2.5, 5, and 10 mM of cocaine induced 16, 45, and 84% of apoptotic figures, respectively, and 6-hour exposure increased apoptosis to 25, 54, and 94%, respectively. This indicated that the drug concentration was more significant than the exposure time (Fig. 3*b*).

Caspase expression and activation in HaCat cells treated with 100 M and 1 mM of cocaine for 1 hour was analyzed by Western blotting (Fig. 4). Although the caspase-3 (32 kDa) and caspase-9 (46 kDa) precursors (inactive form) were consistently detected, the active forms of caspase-9 (35 and 37 kDa) were observed only on 1 mM of cocaine exposure. This result is comparable with HaCat cells treated with 1 M of staurosporine, a known apoptosis inducer.[7] Caspase 8 (57 kDa) precursor was detected, but its activated (18 kDa) fragment was not observed.

DISCUSSION

CIMDL is a rare occurrence despite widespread cocaine abuse by inhalation. Reports on CIMDL involving the sinona-sal tract are rare and its true incidence is unknown, even if isolated septal perforations are reported in 4.8% of cocaine abusers.[1]

The pathogenesis of CIMDL is intriguing. Cocaine, similar to many local anesthetics, interacts with voltage-sensitive Na channels and blocks conduction by altering large Na transients and preventing generation and conduction of nerve impulses. After Ca^2 entry, depolarization of the mitochon- drial outer membrane causes opening of large conductance channels (voltage-dependent anion channels) resulting in volume deregulation that may cause outer membrane rupture within the epithelial layer were observed. The TUNEL and release of cytochrome C and other proteins forming the apoptosome complex.[8] Thus, this event may be triggered by a direct effect of cocaine that activates an intrinsic caspase-9–based apoptotic pathway that occurs when the drug is in contact with the nasal mucosa of abusers. However, CIMDL dramatically affects only a small subpopulation of abusers.

Cocaine also may induce membrane exposure of relevant cell surface proteins on peripheral blood leukocytes, such as

complement and Fc receptors on neutrophils, monocytes, and lymphocytes of cocaine abusers.[9] In some CIMDL patients, ANCA positivity appears as they develop a predisposition for autoantibody formation. Many drugs and infections[10] have been implicated in the induction of autoimmune states, including ANCA-associated vasculitis and most patients have documented superinfections[11] of necrotic tissue lesions. These may give rise to superantigens that maintain antibody production[12] or the antibodies may be a direct, specific response to expressed epitopes resulting in antibodies that cross-react with neutrophil constituents (molecular mimicry).[13] ANCAs reacting with human neutrophil elastase is a diagnostic marker for CIMDL and may discriminate between CIMDL and WG.[14] It has been reported that leukocyte elastase induces epithelial apoptosis[15] and that rats injected with syngeneic rat apoptotic neutrophils develop ANCAs.[16] Although this should be investigated further, it would explain the human neutrophil elastase ANCA positivity found in some subjects or in patients with polyposis or WG but appears to be a characteristic feature of CIMDL patients. For obvious reasons, we had no samples available from individuals that would form the ideal control population, *viz.*, chronic cocaine abusers that do not develop CIMDL.

However, all of the aforementioned and other events do not explain why a destructive lesion starts in a limited population of compulsive abusers. We know that vascular ischemia from excessive cocaine is widely considered to be the major elements in cocaine-induced midline destructive lesions, but we think that this represents only one, albeit important, factor involved in this process. A multifactorial pathogenesis where apoptosis, superinfection, immunologic changes, traumatic effect of cocaine crystals, nose-picking, and superficial necrosis may

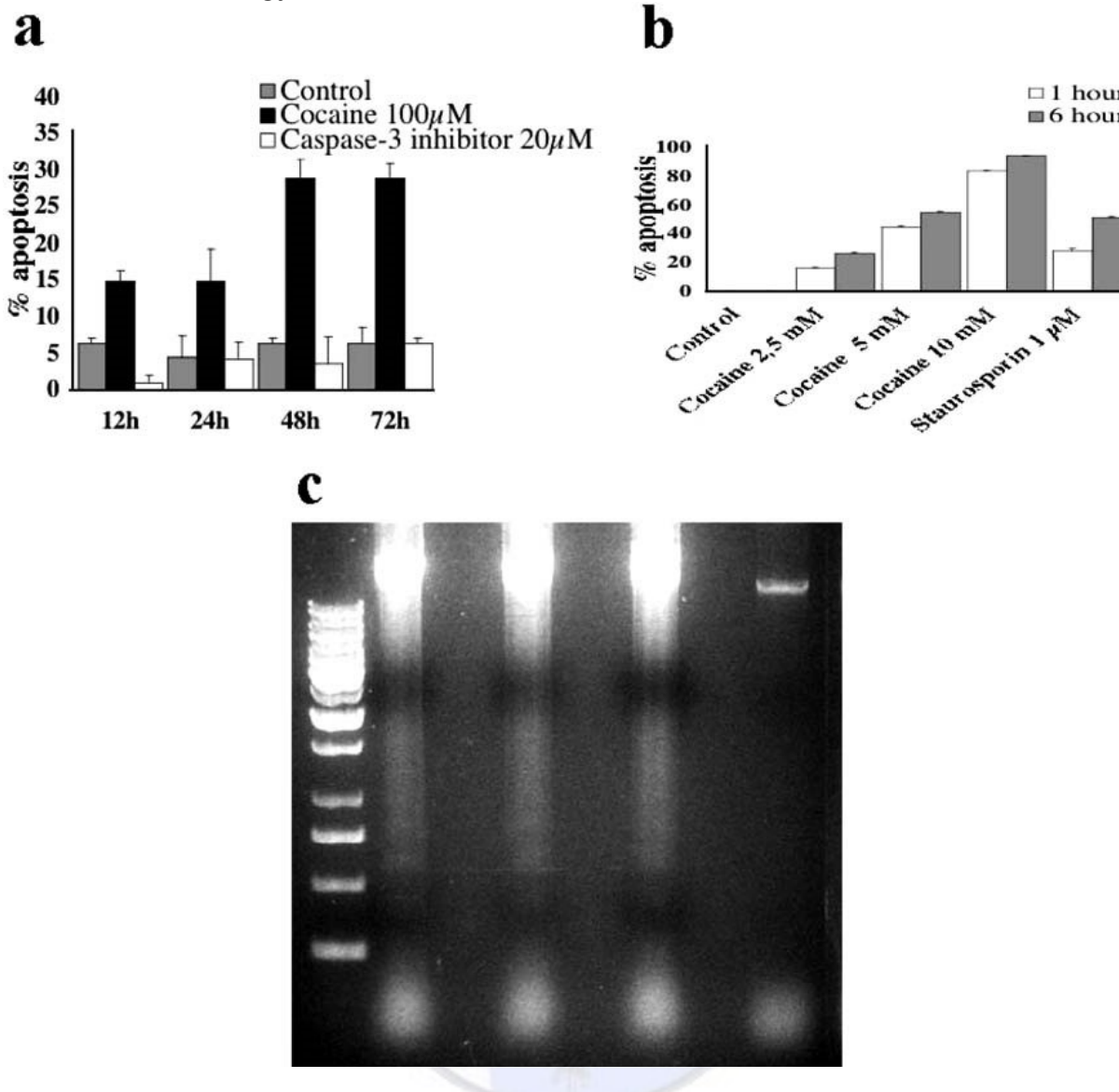

Figure 3. *Effects of cocaine on HaCat cells: (a) percentage of apoptosis of cells treated with 100 M of cocaine w/o 20 M of caspase-3 inhibitor at different times; (b) percentage of apoptosis of cells with different concentrations of cocaine after 1 and 6 hours of treatment; (c) cocaine-induced oligonucleosomal DNA fragmentation on agarose gel.*

CIMDL. From our data, apoptosis is not observed in healthy cocaine crystals. Indeed, after 1 hour we observed that with

induce CIMDL in some individuals. Indeed, the scenario may initiate with cocaine insufflation. After 10–20 minutes, strong vasoconstriction and anesthesia occur and the mucosa is simultaneously altered by the traumatic effects of high cocaine exposure apoptosis is induced, at least *in vitro*. In cocaine abusers, the effect decreases after 1 hour and new administration is compulsively repeated, starting the damaging sequence again. Eventually, patients report epistaxis, headache, hyposmia, and mucopurulent rhinorrhea also, in addition to large nasal scabs, because cocaine interacts with the mucociliary clearance.[17] Abusers often remove scabs improperly, further damaging the nasal mucosa. The progressive mucosal damage, at this point, is multifactorial, because crusting and infection take place on a background of severe and diffuse apoptosis that complicates tissue repair. A complex pathophysiological mechanism may then occur in a few patients that compulsively and continuously inhale high doses of cocaine and probably carry an undefined genetic background. In the small subpopulation of abusers that develop CIMDL, apoptosis induced by cocaine in the nasal mucosa may have a key, added role because nasal mucosa begins anomalous cell death.

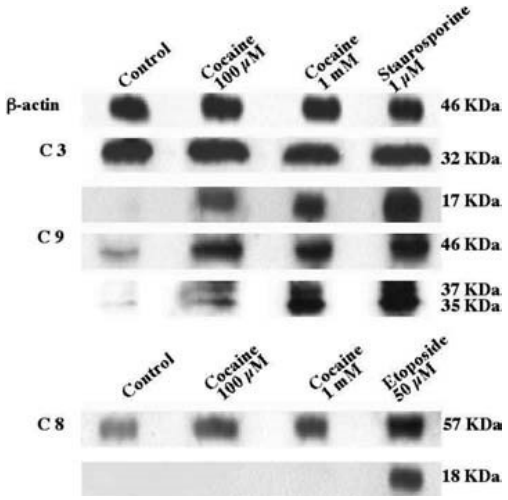

Figure 4. *Western blotting analysis on HaCat cells treated with 100 M and 1 mM of cocaine: casapase-3 and caspase-9, and not caspase- 8 activation detects the mitochondrial-dependent pathway of apoptosis induced by cocaine.*

CONCLUSIONS

Considering the rarity of CIMDL, this potential mechanism may be sustained and, whatever its consequence, the onset of CIMDL may be predicted on the simple basis of a massive tis apoptosis rate performed on a biopsy of nasal mucosa. People at risk, *i.e.*, regular cocaine abusers, should be made aware of the consequences of CIMDL.

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Review

Cocaine-induced midline destructive lesions — An autoimmune disease?☆

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abstract

In Europe it is estimated that around 13 million of adults (15–64 years) have used cocaine at least once in their lifetime. The most frequently used route of administration for the drug is intranasal inhalation, or "snorting", and thus the adverse effects of cocaine on the nasal tract are very common. Habitual nasal insufflations of cocaine may cause mucosal lesions, and if cocaine use becomes chronic and compulsive, progressive damage of the mucosa and perichondrium leads to ischemic necrosis of septal cartilage and perforation of the nasal septum. Occasionally, cocaine-induced lesions cause extensive destruction of the osteocartilaginous structures of nose, sinuses and palate that can mimic other diseases such as tumors, infections, and immunological diseases.

Thorough diagnostic workup, including endoscopic, radiologic, histopathologic and serologic testing is imperative to arrive at the proper diagnosis and to initiate appropriate local and systemic treatment. Positive antineutrophil cytoplasmic antibody (ANCA) test results may be found in an unexpectedly large proportion of patients with CIMDL. In several instances their lesions are clinically indistinguishable from granulomatosis with polyangiitis (Wegener's) limited to the upper respiratory tract. CIMDL seem to be the result of a necrotizing inflammatory tissue response triggered by cocaine abuse in a subset of patients predisposed to produce ANCA, particularly those reacting with HNE. The presence of these HNE-ANCA seems to promote or define the disease phenotype. CIMDL do not respond well to immunosuppressive therapy. Only the consistent removal of persistent stimuli of autoantibody production (cocaine, bacterial superinfections) can halt the disease process, prevent the progression of the lesions and promise success of surgical repair procedures.

Contents

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1. Introduction

With the increasingly widespread illicit use of cocaine, a broad spectrum of clinical pathologies related to this form of drug abuse is emerging [1]. The European Commission on drug abuse stated that 13 million adults (15–64 years) have used cocaine at least once in their lifetime, and some countries have been identified (Spain, UK, Denmark, Ireland, Italy) with a higher prevalence of use among young adults (15–34 years) [2]. The National Household Survey on Drug Abuse reported that in 2008 cocaine was used by 36.7 million Americans [3]. The most frequently used route of administration of powdered cocaine is intranasal inhalation, or “snorting”. Consequently, the adverse effects of cocaine on the nasal tract are common [4].

Habitual insufflation of cocaine powder often causes marked damage to nasal mucosa. However, damage of the underlying perichondrium leading to nasal septum perforation and destruction of the osteocartilaginous scaffold of the nose, sinuses, and palate is much less common (Fig. 1a–d) [5]. These so-called cocaine-induced midline destructive lesions (CIMDL) display a clinical pattern mimicking systemic diseases, with positive antineutrophil cytoplasmic antibodies (ANCA) tests, endoscopic, and radiographic abnormalities chiefly in the nasal septum and turbinates or in other sinonasal sites, as well as marked histopathological changes [6]. In order to provide appropriate treatment, clinicians must recognize these protean disease manifestations and be able to choose diagnostic procedures that allow their differentiation from other inflammatory disease processes, tumors, and infections [5]. The present review focuses on the clinical, radiographic, histopathologic, and serologic features of CIMDL and delineates the potential role of ANCA and apoptosis in their pathogenesis.

2. Clinical presentation

All patients with CIMDL have long standing symptoms including nasal obstruction, epistaxis, and severe facial pain. The most common findings are diffuse necrotizing ulcerative lesions, extensive crusting, and septal perforation [6] (Fig. 1f, g). In more severe cases, the destruction extends to the middle and superior turbinates and the lateral wall of the nose [6] (Fig. 1h). In some patients, hard and soft palate perforations (Fig. 1i, l) may be present and cause dysphagia and nasal reflux, and substantially affect the quality of life [6]. Some individuals with severe destruction develop symptoms caused by propagating infections associated with pseudotumor, proptosis, and diplopia [6]. Symptoms such as fever, malaise, weight loss, as well as arthralgia or myalgia, are typically absent and patients with CIMDL usually don't have any symptoms or laboratory findings indicating a systemic disease at presentation [6].

Magnetic resonance imaging (MRI) represents the most useful first choice of radiographic evaluation of patients with CIMDL [7,12]. MRI can detect areas of an abnormal nasal or paranasal mucosa, which are visualized as hypointensity on T2 and reduced or nonhomogeneous enhancement [5,13]. In cocaine abusers, significant enlargement of the palatine or pharyngeal tonsils associated with small fluid collections within lymphatic tissue has been described [7]. Radiographic signs of otitis media may also be detected. In some

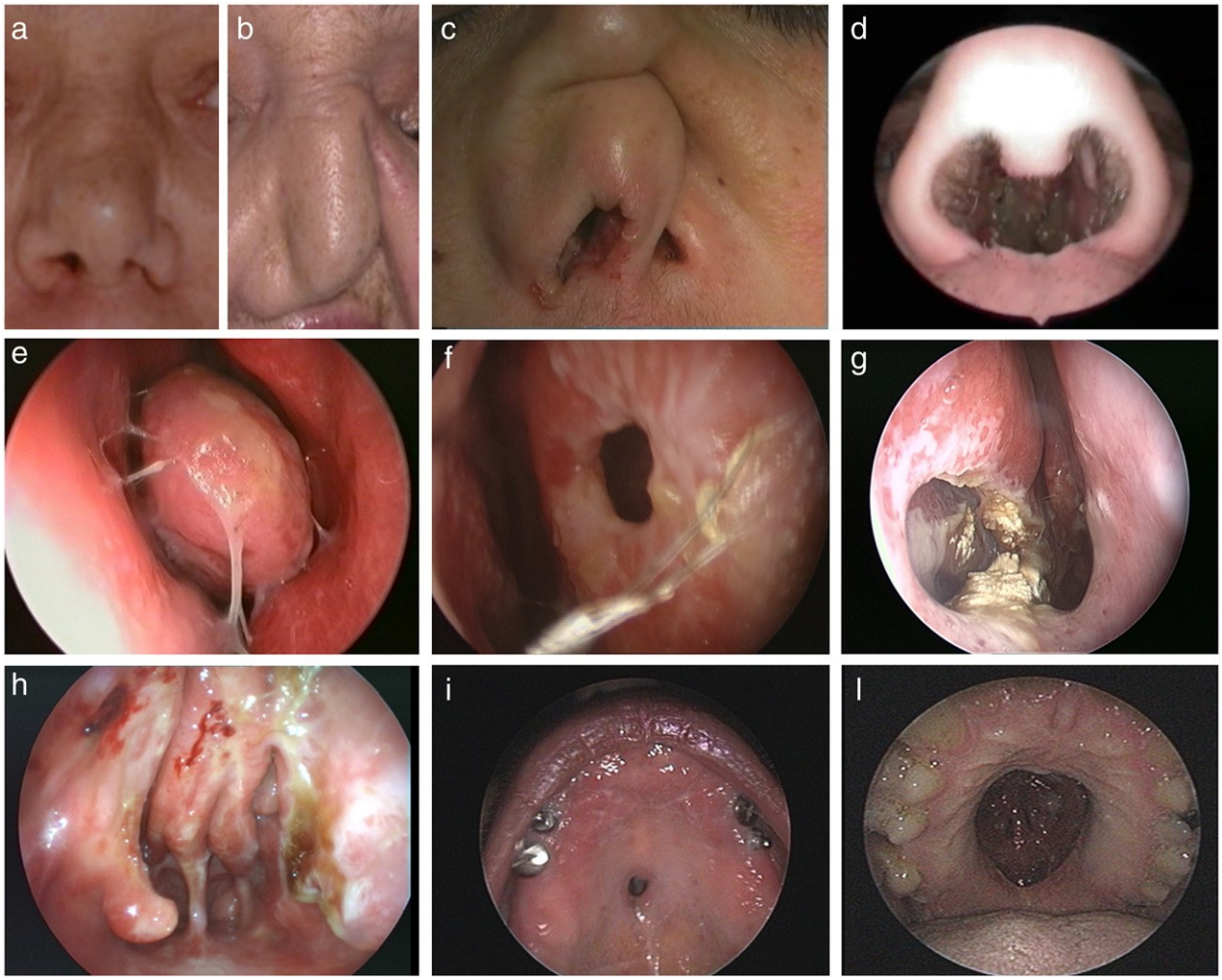

Fig. 1. Different examples of cocaine induced midline destructive lesions. Mild destruction of the nasal dorsum (a) and its evolution after 3 years (b). Destruction of the columella (c). Endoscopic picture of a mild alteration of the mucociliary transport (e) . Endoscopic view of a septal perforation (f). Endoscopic view of a severe septal perforation with crusting and infection (g). Endoscopic view of a complete destruction of the nasal septum and of the inferior turbinates (h). An example of a small (i) and a huge (l) palate perforation taken from the mouth.

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patients, progressive “centrifugal” involvement of the lateral nasal walls and floor with eventual destruction of the entire framework and complete erosion of the hard and soft palate have been noted [5]. 3. Differential diagnosis

Patients often do not readily admit their drug abuse. However, it is possible to evaluate blood, urine and hair for cocaine metabolites to confirm the suspected substance abuse. Midline destructive lesions can be caused by a variety of specific conditions other than cocaine abuse, including infections, neoplasms, sarcoidosis and granulomatosis with polyangiitis (Wegener's, GPA). Their differentiation is important but may at times be very difficult [5]. The correct diagnosis of CIMDL ultimately depends on the clinical history and documentation of drug abuse combined with exclusion of other etiologies [4].

All patients suspected of CIMDL should be evaluated following a comprehensive diagnostic protocol that includes the physical examination of the face, oral cavity, and oropharynx in conjunction with nasal endoscopy. MR and/or CT studies performed with contrast medium are used to determine the presence of mucosal inflammatory lesions as well as cartilaginous and/or bony reabsorption involving sinonasal structures. Multiple biopsy specimens and samples for bacterial and fungal cultures should also be obtained. Infections such as tuberculosis, tertiary syphilis, leishmaniasis, mucormycosis, or infections caused by other organisms can also lead to septal perforation or osteocartilaginous destruction in the nasal cavity. The histopathologic identification of sarcoidosis or lymphoproliferative disorders as cause of midline destructive lesions is usually straight forward. However, the differentiation of CIMDL from limited forms of GPA may be more difficult [5,7].

3.1. Histopathology and serology

The differentiation of CIMDL from GPA with limited nasal or sinus involvement undoubtedly represents the most difficult challenge in individual patients, particularly those who do not readily admit to cocaine abuse. The clinical presentation can provide important clues. The degrees of local destruction are usually much more significant in CIMDL compared to nasal involvement of GPA [7]. In contrast to CIMDL, the isolated nasal involvement in the absence of other disease markers is rare in GPA. In such cases, attempts to obtain a tissue diagnosis should be made. Ideally, multiple biopsy specimens should be obtained from the margins of the pathologic lesion, avoiding the necrotic center which is unlikely to yield any diagnostic tissue.

Unfortunately, the histopathologic differentiation of CIMDL from GPA is difficult for two reasons. First, many histopathologic features are shared between CIMDL and GPA, there are no features specific

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for CIMDL, and the differentiation between the two entities can only be made if the pathognomonic lesions of GPA are found in the biopsy specimen [6]. Second, nasal biopsy specimens are diagnostic of GPA in only about 50% of patients with GPA and nasal involvement [8,9].

Histopathologic features shared between CIMDL and GPA, and thus not allowing their differentiation, include mixed inflammatory infiltrates, microabscesses in vascular walls, perivenulitis, vascular microthrombotic changes, leukocytoclastic vasculitis and fibrinoid necrosis [7]. Only extravascular changes including stromal granulomas with giant cells, microabscesses, and deeply located necrosis are features that seem to be pathognomonic for GPA [7]. Only if these specific features are found in the biopsy specimen can GPA be histopathologically diagnosed, and CIMDL is unlikely. In their absence the histopathologic differentiation between CIMDL and GPA is impossible.

In contrast, the serologic differentiation between CIMDL and GPA is less difficult. Almost all patients with CIMDL have circulating serum anti-neutrophil cytoplasmic antibodies (ANCA) [10]. In CIMDL patients these ANCA are primarily directed against human neutrophil elastase (HNE), generate a perinuclear (P-ANCA) staining pattern on ethanol-fixed neutrophils, and do not react with myeloperoxidase (MPO) [10]. About half of these CIMDL patients may also have coexisting ANCA that react with proteinase 3 (PR3) [10,11]. In contrast, patients with GPA most often have ANCA that generate a cytoplasmic (C-ANCA) staining pattern on ethanol-fixed neutrophils that react with PR3. A minority of patients with GPA have ANCA generating a P-ANCA immunofluorescence pattern and reacting with MPO, or are ANCA negative altogether. HNE-ANCA are usually not found in patients with GPA. Also, the ANCA immune response in GPA is directed against a single target antigen, whereas in patients with CIMDL it is often directed against multiple antigens at the same time [11].

Consequently, in clinical practice both immunofluorescence and antigen specific solid phase assay testing need to be performed in parallel when the differentiation of CIMDL from GPA is sought. The C-ANCA/PR3-ANCA pairing points toward GPA. In contrast, a positive P-ANCA, negative MPO-ANCA, with or without PR3-ANCA should prompt further testing for HNE-ANCA. A positive HNE-ANCA test result points towards CIMDL. Antigen specific solid phase assay testing should not be performed in isolation as a positive PR3-ANCA test result can occur in both GPA and CIMDL and by itself may be misleading.

3.2. Clinical utility of an apoptosis assay

Vascular ischemia from excessive cocaine use is widely considered to be a major element for the development of CIMDL [14–17]. However, if ischemia was the only crucial factor one would expect a much wider CIMDL epidemic in view of the current prevalence of cocaine abuse. Consequently, additional individual host factors must hold an explanation of the enigma of why only a small subset of cocaine abusers develops CIMDL. A multifactorial pathogenesis of CIMDL has been proposed wherein apoptosis, superinfection, immunologic changes, tissue trauma from cocaine crystals, nose-picking, and superficial necrosis all may contribute to the development of CIMDL in some individuals [16,17]. In the small subpopulation of abusers that develop CIMDL, apoptosis induced by cocaine in the nasal mucosa has been identified as potential additional key pathogenic factor [12].

Several investigations on cocaine and apoptosis on cells in vitro and in animal studies have been reported [18–20]. However, there is only one report on increased apoptosis in cocaine abusers [12]. On nasal biopsies, apoptosis is not observed in healthy subjects or in patients with nasal polyposis or GPA; however, apoptosis appears to be a characteristic feature of CIMDL [12]. Apoptotic cells can be identified on tissue sections using a commercially available in situ terminal deoxynucleotidyl transferase-mediated dUTP-digoxygenin nick end labeling (TUNEL) cell death detection kit. Based on our observations, a high level of apoptotic cells seems to be another factor favoring CIMDL over GPA [12]. Therefore, we believe that the application of the TUNEL assay to nasal biopsy specimens may be helpful for the differentiation of CIMDL from GPA in individual situations.

4. The challenges of management of CIMDL

The management and treatment of cocaine abusers represent a real clinical challenge. Once the diagnosis has been established, the nature of the disease process and goals of potential treatment options are explained to the patient. There is no role for immunosuppressive therapy in the management of CIMDL, and patients need to understand that any chance of therapeutic success is entirely dependent on continued abstinence from cocaine. If the abuse continues any therapeutic intervention is doomed to fail. However, medical therapy, prosthesis or surgery can lead to good results if patients reliably stop sniffing [5]. Unfortunately, cocaine abusers are notorious for their poor compliance and dishonesty with physicians [5].

Little is known about the association between personality disorders and neuropsychological test performance in chronic cocaine users [21]. According to Rossellp, there is no robust association between personality profile and neuropsychological test performance [21]. In patients where lesions are very early, discontinuation of drug use may lead to gradual normalization of the nasal mucosa and reversal of the pathologic process [5].

Conservative local treatments such as careful debridement of necrotic tissues and crusts, regular saline douches, and local or systemic application of antibiotic therapy are recommended [5]. These interventions may slow the progression of lesions but cannot reverse the established damage. Obturator prosthesis for palate defects can overcome the oronasal reflux [5], but surgical correction of mucosal or cutaneous defects should be postponed until the lesion is stable.

The decision to treat (or not) as well as the best timing of treatment in relation to the duration of abstinence represents a genuine challenge. The minimum time interval free of sniffing remains controversial, and there is no consensus or large experience to provide guidance. Some authors [22–25] insist on the need to confirm the cessation of cocaine abuse with toxicological tests, ensuring full rehabilitation for 6 months or even 12 months prior to surgery [5,26,27], in order to avoid treatment failure. At the very least, patients must confirm that they are no longer abusing the drug, and the lesion should be relatively stable.

The surgical procedures that are most often requested by patients are septal and palate perforation closure [28–34]. However, some patients ask for aesthetic procedures such as rhinoplasty and closure of naso-cutaneous fistula [22,35,36]. The size and site of lesion influence the type of reconstruction using a local or revascularized free flap [5]. All these interventions should be subject to the same timeframe considerations described above.

5. Pathogenesis

The pathogenesis of CIMDL remains poorly understood. Even the most common form of CIMDL, isolated nasal septal perforation, has been reported for only about 4.8% of habitual nasal cocaine abusers [37]. Given the widespread abuse of nasal cocaine insufflation on the one hand and the relative rarity of CIMDL on the other, differentiating host factors must hold the key to the undoubtedly multifactorial pathogenesis of CIMDL.

The ischemic and direct traumatic effects of cocaine crystals should affect all users similarly [38]. The strong tendency of the damaged nasal mucosa to form tightly adhering scabs prompts affected patients to remove these scabs, often using further damage inducing mechanical methods [39]. Frequency, severity and tenacity of bacterial superinfections of the damaged nasal mucosa may be related to the severity of the underlying mucosal damage as well as individual nasal

hygiene and antimicrobial treatment patterns. Bacterial superinfection has been documented in essentially all patients with CIMDL [7].

What seems to distinguish patients with CIMDL from individuals with similar use patterns without CIMDL, is the presence of ANCA in CIMDL [10]. Even though large screening studies for ANCA in cocaine abusers without pathology are lacking to date, testing of select individuals without pathology who used the same amount of cocaine from the same source as affected CIMDL patients, indicated that the unaffected abusers had no ANCA [10]. Bacterial infections with superantigen producing organisms may lead to ANCA production and long-term persistence in individuals predisposed to autoimmunity [40].

The predominant ANCA type in CIMDL is directed against HNE [10]. The direct effect of HNE-ANCA on the enzymatic activity of HNE or on the binding of HNE on natural inhibitors of HNE does not seem to be factors in the pathogenesis of CIMDL [11]. Yet, the presence of HNE-ANCA may significantly enhance the local inflammatory response to injury. This is supported by a murine model of PR3-ANCA [41]. The spectrum of proteolytic substrates and inhibitors of murine PR3 is very similar to that of human HNE, but quite dissimilar from that of human PR3 [42]. In the presence of anti-murine PR3 antibodies wild-type mice had a significantly more severe inflammatory response to a localized inflammatory stimulus than wild-type mice which received control antibodies [41].

Moreover, cocaine seems to induce apoptosis of the respiratory epithelial cells and possibly also of inflammatory cells present in the nasal passages [12]. The apoptosis inducing effects of cocaine are dose and time dependent [12]. Thus, individuals with the same use patterns should be affected similarly. However, it is possible that the fate of apoptotic cells is quite different in the presence of ANCA. Several in vitro studies suggest that ANCA modify the clearance of apoptotic cells. Opsonization of preaptotic cells by ANCA has been associated with an increased production of inflammatory cytokines by phagocytosing macrophages [43]. Moreover, preapoptotic cells have a decreased cell surface expression of phosphatidylserine (the recognition signal for macrophages) in the presence of ANCA [44]. Thus, in the presence of ANCA the non-inflammatory clearance of apoptotic cells by macrophages may be perturbed in favor of inflammation and necrosis, and cocaine induced apoptosis may lead to more significant CIMDL promoting inflammation and necrosis in the presence of ANCA.

In summary, CIMDL seem to be the result of a necrotizing inflammatory tissue response triggered by cocaine abuse in a subset of patients predisposed to produce ANCA, particularly those reacting with HNE. The presence of these HNE-ANCA seems to promote or define the disease phenotype. This is similar to anti-granulocyte antibodies being associated with granulocytopenias and anti-phospholipid antibodies being associated with microthrombotic cutaneous vasculopathies emerging as autoimmune phenomena related to levamisole which is increasingly used as an additive to street cocaine [1]. None of these cocaine abuse associated autoimmune phenomena seem to respond well to immunosuppressive therapy. Only the consistent removal of persistent stimuli of autoantibody production (cocaine, levamisole and bacterial superinfections) can halt the disease process, prevent the progression of the lesions and promise success of surgical repair procedures.

Take-home messages

- Cocaine-induced midline destructive lesions (CIMDL) are part of spectrum of cocaine abuse associated autoimmune phenomena.
- The differential diagnosis of CIMDL includes other diseases associated with similar midline tissue pathology, particularly granulomatosis with polyangiitis (GPA).
- ANCA serology can complicate the differential diagnosis, but the presence of ANCA directed against several different autoantigens at the same time, and the presence of ANCA directed against HNE set CIMDL apart from GPA.
- A high apoptotic cell index on biopsy specimens from patients with CIMDL may also help to differentiate CIMDL from GPA.
- The benefit of immunosuppressive therapy is limited in CIMDL, and abstinence and careful debridement in combination with appropriate antimicrobial therapy form the basis for any therapeutic success.

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Gene Expression Analysis in Patients with Cocaine-Induced Midline Destructive Lesions

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Abstract: Background and Objectives: Cocaine users may present with positive antineutrophil cytoplasmic antibodies (ANCA) and severe midline destructive lesions (CIMDL) which are histologically characterized by massive apoptosis. However, histopathological and laboratory studies suggest that autoimmunity may not be the main pathogenic driver. We analyzed gene expression both in cell lines of nasal mucosa exposed to cocaine and in CIMDL patients to determine whether genetic predisposition might cause such lesions, which are observed in a minority of cocaine abusers. Materials and Methods: The genetic expression profile of nasal mucosa exposed to cocaine was analyzed. Rare variants of expressed genes were searched in patients with CIMDL using exome sequencing and bio-informatics. Results: We identified 462 genes that were induced by cocaine, mainly related to apoptosis and autophagy in response to oxidative stress. Under the hypothesis that genes linked to the phenotype are also induced by cocaine itself, a rare variants burden test was performed to select genes that were significantly enriched in rare mutations. Next, 11 cocaine abusers with CIMDL and no other relevant medical comorbidities underwent exome sequencing, and 12 genes that were significantly enriched in the burden test and present in at least 10 patients were identified. An in-depth analysis of these genes revealed their involvement in apoptosis, tissue homeostasis, autophagy, and response to oxidative stress. Conclusions: Oxidative stress and rare genetic alterations in the response to reactive oxygen species, apoptosis, autophagy, and tissue regeneration are plausible drivers of damage affecting nasal mucosa exposed to cocaine crystals and, consequently, the pathogenic mechanism behind CIMDL.

Keywords: paranasal sinus disease; craniofacial region; chronic disease; cocaine; CIMDL

1. Introduction

Cocaine is the most commonly used illicit stimulant drug in Europe [1]. Estimates of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) indicate that about 2.6 million young adults (aged 15 to 34) used cocaine during the previous year [1]. Intranasal cocaine abuse is known to cause damage to nasal mucosa, along with potentially fatal systemic effects and addiction [2,3]. In some habitual cocaine users, however, damage to nasal structures can extend to the underlying osseous and cartilaginous structures of the nose, resulting in cocaine-induced midline destructive lesions (CIMDL) (Figure 1) [2,4]. While the exact prevalence of CIMDL is unknown, a search of the US National Library of Medicine (PubMed) online database from 1 January 1982 to 31 December 2019 found 114 reports describing roughly 200 patients; however, this number is in stark contrast with data from the US Substance Abuse and Mental Health Services Administration, which estimated that in 2018 about 5.5 million US residents used cocaine [5]. In fact, if CIMDL were simply the last stage of chronic cocaine-induced mucosal damage and erosion, it would be expected that these lesions would be much more prevalent among habitual users, even if the possible role of cutting substances is taken into account. Moreover, our

clinical experience suggests that individuals exposed to the same drug batches at similar doses do not necessarily develop lesions with the same frequency or severity [1].

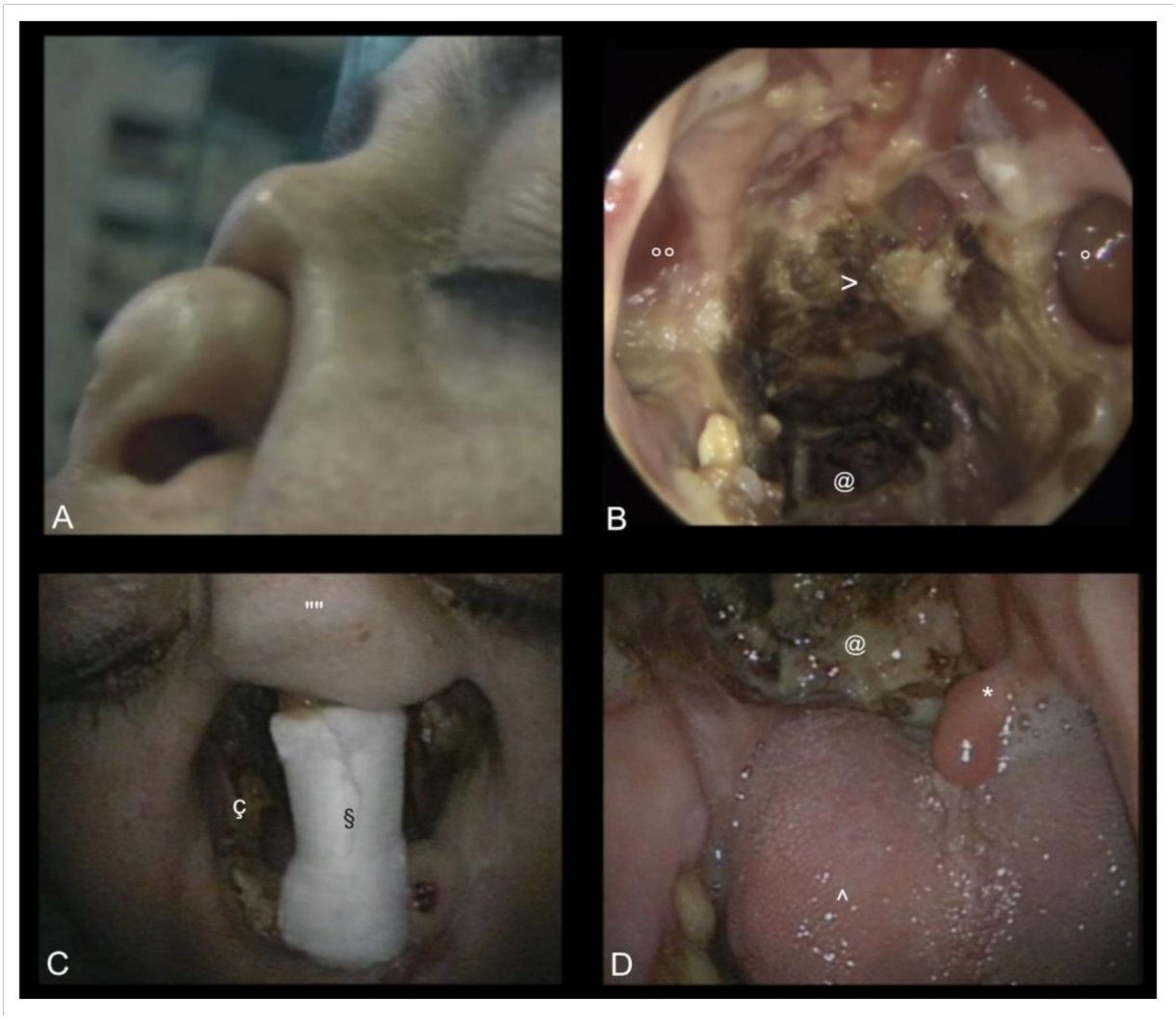

Figure 1. The picture shows the extent of cocaine-induced damage to the nasal dorsum (**A**), columella (**C**, §), and superior lip (**C**; ç indicates erosion of the piriform aperture; "" marks the remaining nasal dorsum). Pictures (**B**,**D**) show the endoscopic endonasal view of the oro-nasal cavity of the case illustrated in picture (**C**), which shows the total erosion of nasal septum, inferior, middle, and superior conchas, hard palate, sub-total erosion of soft palate (* shows the remaining uvula), exposure of the skull base (@ and >), as well as opening of the maxillary sinuses (° and °°).

Since the clinical appearance of CIMDL overlaps with upper respiratory tract granulomatosis with polyangiitis (GPA), autoimmunity has been suggested as a possible cause [2]. However, histopathological and laboratory studies conducted to date suggest that cocaineinduced apoptosis and not autoimmunity may be the main driver of those lesions [2]. Moreover, cocaine is known to induce the expression of genes with a role in response to oxidative stress and DNA damage [6,7]. Based on these findings, to further elucidate the pathogenesis of CIMDL we analyzed the genetic expression of nasal mucosa samples exposed to cocaine. Expressed genes were then examined in patients with CIMDL using whole exome sequencing and bioinformatics analysis to identify the main biomolecular mechanisms involved in CIMDL and confirm the expression of genes related to apoptosis and oxidative stress response.

Secondly, considering the discrepancy between the number of cocaine abusers and the relative low frequency of CIMDL we hypothesized that genetic predisposition could play a role in CIMDL expression. Therefore, we tried to find rare variants of the identified cocaine-induced genes that could be linked to the destructive effect of cocaine abuse.

We then attempted to verify the expression of the identified rare variants in a sample of CIMDL patients.

2. Materials and Methods

2.1. Cell Culture

The nasal epithelial immortalized cell line RPMI 2650 from ATCC® was used as an in vitro nasal model.

2.2. Reagents

Cocaine base (LGC, Teddington, UK) was prepared as 50 mM solution (50% Phosphate Buffered Saline (PBS)/50% ethanol). Staurosporine, a known inducer of apoptosis, was employed as a positive control, prepared as 15 mM in Dimethyl Sulfoxide (DMSO).

2.3. Experimental Design

In order to determine the optimal time window and treatment conditions, we performed a preliminary experiment using two different treatment times (1 and 6 h), different concentrations of cocaine (0.1 µM, 1 µM, 2 µM, 3 µM, 4 µM, 5 µM) and staurosporine (1 nM, 5 nM, 10 nM), along with two negative controls (cell culture medium alone, and cell culture medium with 50% of PBS/ethanol, 1:1). After the incubation period, cells were washed and incubated without drug for 24 or 48 h, respectively. Starting from 3 µM cocaine, massive apoptosis was observed in both short and long treatment periods (data not shown).

The experiment was then repeated under the following conditions: incubation for 6 h at 37 °C in the presence of either cocaine (0.1 µM, 1 µM, 2 µM, 3 µM, 4 µM, 5 µM) or staurosporine (1 nM, 5 nM, 10 nM), and two negative controls (see above), followed by washing, cell harvesting, and RNA extraction after 24 h. All cells were plated in duplicate (technical replicates) for each time point to determine toxicity and to extract RNA. All experiments were done in triplicate.

2.4. RNA Extraction and High-Throughput Sequencing (HTS)

RNA from treated cells and control samples was extracted using the RNeasy Mini Kit ® (Qiagen, Hilden, Germany). Quality and quantity of total RNA were evaluated by running samples onto Tapestation 4100® (Agilent, Santa Clara, CA, USA) and Qubit® (Thermofisher, Waltham, MA, USA), respectively. Libraries were prepared for HTS sequencing using the TruSeq® mRNA stranded kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol starting from 100 ng of total RNA. Sequencing was performed using Novaseq 6000® (Illumina, San Diego, CA, USA) in SE mode, generating in average 30 M reads per sample, 100 nt long.

2.5. RNA-Seq

Read tags were pseudo-aligned to GENCODE transcripts v27 [8] using Kallisto v0.44.0 (Pachter Lab, Pasadena, CA, USA). [9] Transcripts were summarized to genes using the tximport [10] package (Bioconductor, Buffalo, NY, USA). Differential expression was evaluated using limma (Bioconductor, Buffalo, NY, USA) [11] interpolating the dose of chemical (cocaine or staurosporine) with a spline curve using two degrees of freedom. Genes induced by medium (PBS + ethanol) were estimated by a comparison with untreated cells. *p*-values were corrected using qvalue package (Bioconductor, Buffalo, NY, USA) [12]. Genes were considered significant at $q < 1 \times 10^{-3}$. Genes that were found to be regulated by cocaine but not by staurosporine or PBS-ethanol were considered for investigation.

2.6. Exome Sequencing

Exome sequencing was performed on blood samples drawn from CIMDL patients who had been referred to the Otorhinolaryngology Department of San Raffaele Hospital (Milan, Italy). All patients gave informed consent and the study was conducted according to the principles of the revised Declaration of Helsinki, in compliance with Good Clinical Practice

and ethical standards, and was approved by local ethics committee (Comitato Etico dell'Ospedale San Raffaele).

Read tags were aligned to reference genome hg19 using BWA MEM (Illumina, San Diego, CA, USA) [13] and duplicated reads were identified using Samblaster (GitHub, San Francisco, CA, USA) [14]. Variant calling was performed using Freebayes [15] Variant Call Format (GitHub, San Francisco, CA, USA) was annotated for functional impact using snpEff (Paolo Cingolani, Arlington, MA, USA) [16]. Variants were filtered for quality (QUAL > 1, RPL > 1, RPR > 1, SAF > 0, SAR > 0, MQM > 50, MQMR > 50) and for impact on the protein sequence (MODERATE or HIGH impact). Variants were further filtered for allele frequency lower than 1% in dbSNP v146 (National Center for Biotechnology Information, Bethesda, MD, USA) (coded allele frequency < 0.01). Burden test was performed using TRAPD (GitHub, San Francisco, CA, USA) [17] using the Exome Aggregation Consortium data as controls. *p*-values were corrected by False Discovery Rate [18].

3. Results

We performed RNA sequencing to identify genes that were regulated by incubation of cocaine with cultured cells. To do so, we tested increasing concentrations of cocaine (0.1 µM, 1 µM, 2 µM, 3 µM, 4 µM, 5 µM); we also tested increasing concentrations of staurosporine (1 nM, 5 nM, 10 nM) as control for apoptosis. Indeed, increasing the concentration of cocaine inhibited cell growth ($p = 1.29 \times 10^{-7}$), occurring at cocaine concentrations as low as 3 µM (Figure 2).

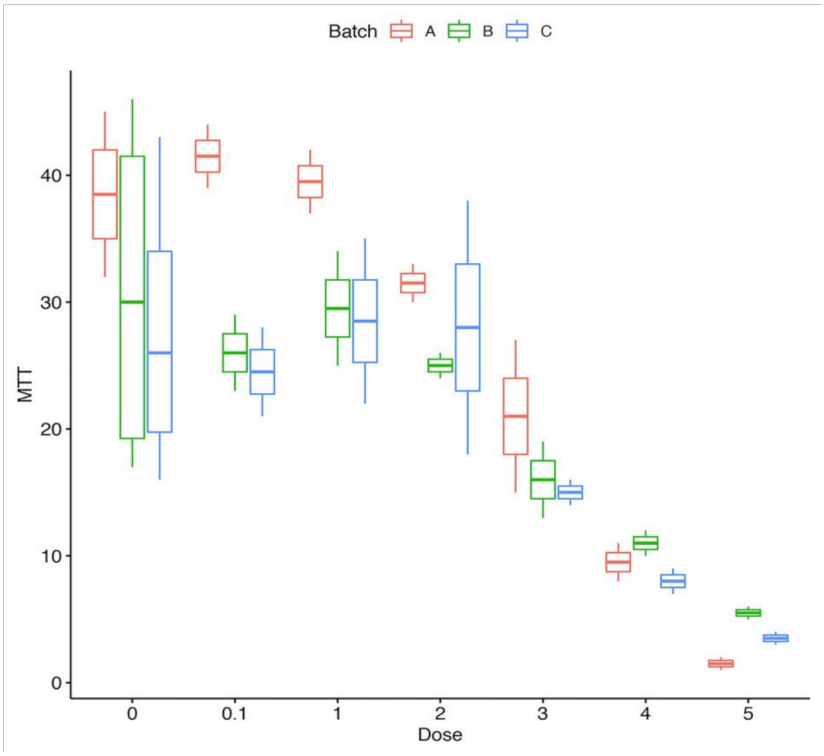

Figure 2. Effect of cocaine on cell growth. The boxplot shows the cell vitality, measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), at increasing dosage of cocaine. We present data for three experiments, each in triplicate (A, B, and C).

We identified 462 genes that were induced by cocaine in a specific manner (Figure 3A). Analysis of Gene Ontology terms showed an enrichment in processes and compartments mainly related to apoptosis and autophagy/lysosomal activity (Figure 3B). Under the hypothesis that genes linked to the clinical phenotype are also induced by cocaine, a rare variants burden test was performed to select genes that were significantly enriched in rare mutations compared to a control population.

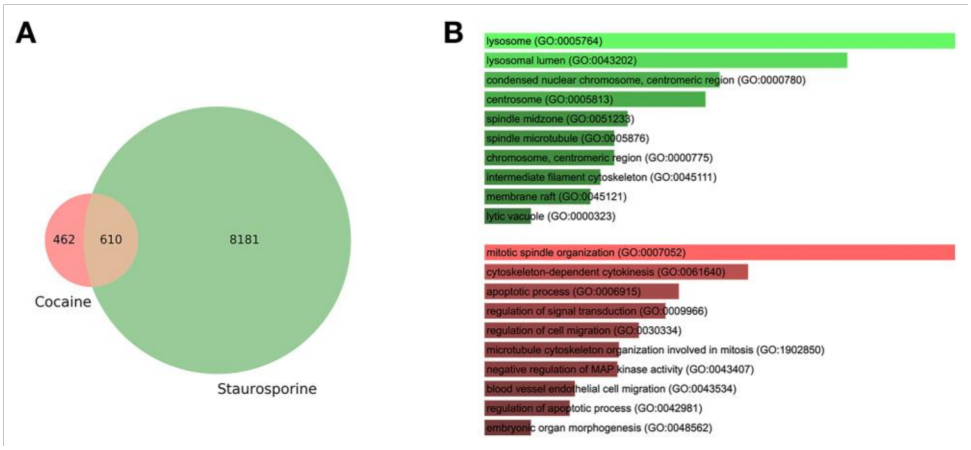

Figure 3. (**A**) Differentially expressed genes upon cocaine administration. The Venn diagram shows the number of genes that are regulated at increasing dose of cocaine or staurosporine. Genes were selected at $q < 1 \times 10^{-3}$; out of 1072 genes, only 462 were found specific for cocaine. (**B**) Gene Ontology analysis. The bar chart shows the top 10 GO:BP (gene ontology:biological process) categories enriched for the genes specifically induced by cocaine. A large fraction of terms is related to apoptosis.

Following this, in order to identify genes possibly associated with CIMDL we performed exome sequencing on a set of 11 cocaine abusers with CIMDL (six females and five males). Age and degree of facial destruction caused by CIMDL are reported in Table 1.

Table 1. Age, gender, and involved anatomical structures of the CIMDL patients included in the study.

Case Number	Gender	Age (Years)	Involved Anatomical Structures
1	Female	41	Nasal septum, middle, and inferior turbinates
2	Female	25	Nasal septum, middle, and inferior turbinates, lateral nasal wall, hard palate.
3	Female	38	Nasal septum, inferior turbinates
4	Male	45	Nasal septum, middle, and inferior turbinates, lateral nasal wall, soft palate.
5	Female	40	Nasal septum, inferior turbinates
6	Male	33	Nasal septum, middle, and inferior turbinates
7	Male	40	Nasal septum, middle, and inferior turbinates
8	Male	66	Nasal septum, middle, and inferior turbinates, lateral nasal wall, hard palate.
9	Male	39	Nasal septum, middle, and inferior turbinates
10	Female	32	Nasal septum, inferior turbinates
11	Female	31	Nasal septum, middle, and inferior turbinates, lateral nasal wall.

All patients sought medical care for CIMDL-related symptoms and had no other relevant medical comorbidities. Twelve genes that were significantly enriched in the burden test and present in at least 10 patients were identified: AHNAK, C1orf116, CACHD1, FBN1, IQGAP2, OSGIN1, PARP4, PDLIM5, PPP1R15A, PVR, TBC1D2, and ZNF469. Interestingly, all genes were found to be induced and none repressed by cocaine, suggesting a possible mechanism of loss-of-function. An in-depth analysis of these genes revealed their involvement in apoptosis, tissue homeostasis, autophagy, and response to oxidative stress (Table 2).

Table 2. Characteristics and processes of the twelve genes related to CIMDL. Case HET: Number of individuals carrying at least one heterozygous qualifying variant in the gene; Case HOM: Number of individuals carrying at least one homozygous qualifying variant in the gene; Case AC: Total Allele Count of qualifying variants in the gene; Control HET: Approximate number of individuals carrying heterozygous qualifying variants in the gene; Control HOM: Number of individuals carrying homozygous qualifying variants in the gene; Control AC: Total AC for the gene; *p*-value: *p*-value under the dominant model; Adjusted *p*-value: *p*-value corrected with Benjamini-Hochberg procedure; Processes: main function associated to gene.

Gene SYMBOL	Case HET	Case HOM	Case AC	Control HET	Control HOM	Control AC	*p*-Value	Adjusted *p*-Value	Processes
AHNAK	12	12	154	0	0	0	2.20×10^{-49}	2.02×10^{-45}	Cell adhesion
C1orf116	12	11	140	154	0	154	1.34×10^{-31}	1.14×10^{-27}	Wound healing
CACHD1	12	12	140	97	0	97	6.89×10^{-34}	5.94×10^{-30}	Apoptosis
FBN1	12	12	141	226	1	228	1.20×10^{-29}	1.02×10^{-25}	ECM formation, Cell adhesion
IQGAP2	12	12	281	135	1	137	3.21×10^{-32}	2.75×10^{-28}	Cytoskeleton
PARP4	12	11	374	1291	4	1299	1.08×10^{-20}	8.78×10^{-17}	Cell growth
PDLIM5	12	12	143	1267	10	1287	9.17×10^{-21}	7.43×10^{-17}	Cytoskeleton
PVR	12	12	138	0	0	0	2.20×10^{-49}	2.02×10^{-45}	Cell adhesion
ZNF469	12	12	443	321	0	321	6.98×10^{-28}	5.90×10^{-24}	Collagen formation
OSGIN1	11	4	171	848	9	866	7.88×10^{-23}	6.46×10^{-19}	Oxidative Stress, Apoptosis.
PPP1R15A	10	2	97	854	5	864	8.11×10^{-23}	6.64×10^{-19}	Apoptosis, Oxidative stress, wound healing.
TBC1D2	10	1	58	1435	10	1455	1.94×10^{-17}	1.54×10^{-13}	Autophagy

4. Discussion

Intranasal cocaine is a well-known irritating factor for the nasal mucosa, but in only a small percentage of habitual abusers the damage extends to the underlying structures causing CIMDL. Several studies have been conducted trying to understand the predisposing factors of these type of lesions, with no common consensus achieved [2,3]. To date, several aspects make research on CIMDL complex, namely the unreliability of abusers' reporting, the difficulty in determining the exact substance concentration, length of use, frequency, and quantity administered [4,7]. Nonetheless, our clinical experience and evaluation of current epidemiological data on cocaine abuse suggest that, for unknown reasons, only a minority of abusers develop CIMDL. Specifically, considering the supposed rarity of CIMDL, it is possible that genetically determined predisposing factors could play a pivotal role in its genesis.

This study is the first to investigate gene expression in CIMDL and found out that exposure of nasal epithelium to cocaine induces a diverse array of genes mainly related to apoptosis and autophagy/lysosomal activity, confirming that cocaine promotes apoptosis in exposed tissues [2,3,7].

In our CIMDL patient cohort 12 cocaine-induced genes bearing rare variants have been identified with a frequency greater than 80%, but none in all patients. As a consequence, a monogenic mechanism in CIMDL predisposition can be likely excluded. However, these observations do not rule out the possibility of genetic predisposition, which could still be present with involvement of multiple genes along common response pathways.

The genes analyzed are involved in different cellular functions such as apoptosis and autophagy, tissue regeneration, cell proliferation, collagen integrity, and DNA damage response [19–22]. These findings support current knowledge about cocaine-induced tissue damage, which most probably results from oxidative stress and consequent apoptosis [7,23].

Nonetheless, in spite of already published evidence, widespread misconception remains about the pathogenesis of CIMDL, often attributed to mechanical irritation by cocaine crystals and hypoxic necrosis secondary to vasoconstriction, which are still mentioned in the literature as putative pathogenic mechanisms [24].

Among the 12 rare gene variants identified, 5 (OSGIN1, CACHD1, PPP1R15A, TBC1D2, PARP4) are related to autophagy and/or apoptosis. The latter is known to be induced by cocaine through generation of reactive oxygen species (ROS) that interact with

DNA, mitochondrial membranes, and endoplasmic reticulum (ER), [6,23] and our findings confirm the importance of these processes in the pathogenesis of CIMDL [7,23]. Among the described genes, OSGIN1 and PPP1R15A appear to be the most closely associated with CIMDL, due to their direct implication with apoptosis. On the other hand, CACHD1, TBC1D2, and PARP4 could still be implicated, but their involvement in cell death and oxidative stress response appears less direct [25–27].

Other rare gene variants that were identified are not related to the response to oxidative stress, apoptosis, and autophagy, but rather to cell proliferation and tissue healing. Understandably, in the presence of ROS-related damage, not only pro-apoptotic or proautophagic molecular mechanisms play a role in the elimination of damaged structures and cells, but also regeneration is stimulated in response to injury and apoptosis [28] and could play an important role in the development of the CIMDL phenotype.

The genes identified, namely AHNAK, C1orf116, FBN1, IQGAP1, PDLIM5, ZNF469, and PVR, are less decisively linked with CIMDL due to the absence of direct association with apoptosis or cell death. Additionally, it is difficult to hypothesize a clear mechanism of involvement in CIMDL pathogenesis for genes whose functions are poorly characterized, such as AHNAK [29] and C1orf116 [19].

The histological features of apoptosis are peculiar characteristics of CIMDL and represent one of the main tools useful to make a correct differential diagnosis with GPA, autoimmune pathology with similar clinical presentation compared to cocaine induced nasal lesions. In fact, both these conditions present histological features like mixed inflammatory infiltrates, microabscesses in vascular walls, perivenulitis, vascular microthrombotic changes, leukocytoclastic, vasculitis, and fibrinoid necrosis, [30,31] but only CIMDL presents massive apoptosis. In addition, the absence of extravascular changes (e.g., stromal granulomas with giant cells, microabscesses, and deeply located necrosis) peculiar to GPA, gives an additional clue for a correct differential diagnosis. Unfortunately, these histological differences are not always present and, in addition, CIMDL and GPA can share serological positivity for c-ANCA or p-ANCA [32,33]. These similarities are an additional challenge that can lead to incorrect diagnosis. However, recent studies have demonstrated that ANCA specificity for human neutrophil elastase (HNE), is peculiar of CIMDL and can be used, in addition to the histological feature of apoptosis, as a diagnostic tool [3]. At present, it is still unclear whether ANCA are active participants in the pathogenesis of CIMDL or, more probably, an epiphenomenon [2,3]. While it is theoretically possible that inflammation and immunity play a role in the development of CIMDL, it is unlikely to be the sole pathogenic mechanism, considering the experimental evidence gathered in the present study.

This study presents some limitations. Firstly, while CIMDL patients, due to the progressive nature of their condition, [2] are likely to seek medical attention at some point, it is more difficult to study cocaine abusers without this condition. Indeed, repeating the same experiments on abusers without CIMDL might prove useful to identify relevant gene variants. Secondly, the rare variants related to addiction could co-segregate with those associated with CIMDL. Thirdly, it must be considered for all the selected genes that the increased frequency of rare variants in our population could be related to unknown selection bias. However, considering the known pathogenic mechanisms involved in CIMDL, it is reasonably possible to differentiate between variants related to each of the conditions. Fourthly, additional concern could rise if considered the “real” street dose of cocaine which is generally unknown due to the multiple times cut in the selling pipeline. However, our experiment deals with different concentration of cocaine, reducing, as much as possible, the influencing variability of cocaine dose. Fifthly, the gene expression of the nasal epithelium is limited to the acute effects of cocaine on the mucosa, while gene expression and immunological response resulting from chronic exposition to cocaine have not been investigated; indeed, the chronic effects of cocaine on nasal mucosa could be the subject of further studies.

5. Conclusions

Our study demonstrated induced expression of a diverse array of pro-apoptotic genes in nasal epithelium exposed to cocaine. When considering the cohort of CIMDL patients, rare variants of 12 genes related to apoptosis, autophagy, tissue regeneration, cell proliferation, collagen integrity, and DNA damage response were expressed. Our findings, along with evidence from other studies, support the notion that oxidative stress is a crucial driver of damage affecting nasal mucosa exposed to cocaine crystals and, consequently, the most likely pathogenic mechanism of CIMDL.

However, our experiments could not determine the relative importance of each expressed gene or molecular pathway in CIMDL and were limited by lack of data on disease prevalence, small sample size and absence of a control group of cocaine abusers without CIMDL. Determination of CIMDL prevalence and future experimental models with gene silencing could be helpful to delineate the molecular pathways relevant to CIMDL and elucidate its pathogenesis and the possible role of genetic predisposition.

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Institutional Review Board Statement: The study was conducted according to the principles of the revised Declaration of Helsinki, in compliance with Good Clinical Practice and ethical standards, and was approved by local ethics committee (Comitato Etico dell’Ospedale San Raffaele, approval number 2-24102013; date of approval 24 October 2013).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: Data are available from the authors upon reasonable request.

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Dacryocystorhinostomy: Evolution of endoscopic techniques after 498 cases

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Abstract

Introduction: Endoscopic dacryocystorhinostomy is a well-known surgical practice used to treat nasolacrimal duct obstruction and widely considered as a valid alternative to external approaches.

Purpose: We present a retrospective case series of 498 endoscopic dacryocystorhinostomies on 401 patients, from July 2004 to May 2018, at the Department of Otolaryngology, San Raffaele Hospital, Milan, Italy.

Methods: Of the 498 procedures, 426 were unilateral and 72 were bilateral dacryocystorhinostomy. All patients underwent routine preoperative workup including fluorescein test (Jones test 1–2), probing and irrigation of the lacrimal way, nasal endoscopy, and maxilla-facial computed tomography scan. Surgical technique was based on nasal endoscopic dacryocystorhinostomy followed by positioning of a Catalano's silicone stent, which was left in place for about 3 months. Anatomical success was defined as a patent ostium on irrigation, whereas functional success was defined as free lacrimal flow on functional test and resolution of epiphora.

Results: Anatomic success was achieved in 91.54% cases in primary dacryocystorhinostomy and in 89.36% after revision, whereas functional success was obtained in 90.4% in primary and 85.1% in secondary dacryocystorhinostomies. After a second revision of endoscopic dacryocystorhinostomy, anatomical success was achieved in 90.1% and functional success in 88.7% of procedures.

Conclusion: Our results confirm that endoscopic dacryocystorhinostomy can be considered as a valid surgical approach to primary nasolacrimal duct obstruction and revision cases. The key aspects in achieving functional and anatomical results are meticulous surgical procedure and precise follow-up.

Keywords

DCR, nasolacrimal duct obstruction, endoscopic dacryocystorhinostomy

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Introduction

Nasolacrimal duct obstruction (NLDO) is a common pathology that prevents natural eye to nose tears, leading to the common symptom of epiphora; however, the intensity of symptomatology and objective findings are not always strongly correlated.

The causes of obstruction in NLDO may range from congenital to acquired: primary acquired nasolacrimal duct obstruction (PANDO) is commonly caused by unknown fibrosis or inflammation and is more common in adult women, whereas secondary acquired NLDO can occur, for example, because of trauma, surgery, or neoplasms.[1]

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With the introduction of advanced fiberoptic endoscopes, nasal endoscopic dacryocystorhinostomy (ENDDCR) has become a well-tolerated and successful procedure for NLDO.[2] With this surgical approach, a permanent connection between the lacrimal sac and the nose is made without cutaneous incision or disruption of the lacrimal pump.[3] Additional advantages of END-DCR are reduced operative time, post-surgical morbidity, and early recovery.[4]

The standard surgical endonasal approach consists of the creation of the largest possible osteotomy and sac marsupialization, which is associated with high short-term success rates. Nevertheless, long-term success rates may range between 81% and 96%,[3,5–8] which is comparable to that of a traditional, external approach.[3,9,10]

The aim of this study is to report our clinical and surgical experience on 498 consecutive cases of END-DCR, discussing clinical and surgical outcomes and follow-up.

Materials and methods

In this retrospective study, we included all patients who underwent END-DCR for NLDO between July 2004 and May 2018 at the Department of Otolaryngology, San Raffaele Hospital, Milan, Italy.

Informed consent was obtained from each patient for treatment and use of de-identified clinical data for study purposes. We obtained approval from the institutional review board (IRB) of San Raffaele Hospital for this clinical review study, which was conducted according to the ethical standards established in the 1964 Declaration of Helsinki, as revised in 2000.

All patients had a clear diagnosis of NLDO made by multidisciplinary agreement between an otolaryngologist and ophthalmologist with unanimous agreement on the site of obstruction. Multidisciplinary diagnostic workup was performed according to the preoperative analyses proposed by Trimarchi et al.[1] In particular, it is based on functional fluorescein test (Jones test 1 and 2), lacrimal probing, and irrigation; as part of the workup, after clinical evaluation, nasal endoscopy and maxilla-facial computed tomography (CT) scan were carried out to assess nasal anatomy. Patients with immunologic deficiency, congenital pathology, canalicular abnormalities, and nasal dysmorphisms were excluded from the study. All patients underwent END-DCR by the same otolaryngologist and ophthalmologist.

Surgical technique

The surgical aim was to create a patent communication between the lacrimal sac and nasal cavity. Under general anesthesia, using a 30° endoscope, the maxillary line was identified and a mucosal flap was raised posteriorly to expose the lacrimal bone. The incision started at the middle turbinate axilla, continuing 5 mm anteriorly, then a cranio-caudally direction, parallel to the maxillary line, was taken until insertion of the inferior turbinate; finally, the incision continued 8 mm posteriorly. The lacrimal bone was then palpated to detect the junction with the frontal process of the hard palate. Using a powered instrument, the lacrimal bone and the lower part of the frontal process were removed. While the medial wall of the lacrimal sac was exposed, a Bowman's probe was used by an ophthalmologist to tent the medial sac wall passing through the inferior punctum. The tip of this instrument was used as a guide to make a vertical incision of the lacrimal sac, and an anterior and posterior releasing incisions were then made, creating an "H" shape. The horizontal cuts allowed for creation of an "open book" flap that was rolled out on the lateral wall of the nose. The ophthalmologist then dilated the upper and lower puncta using a Catalano stent that was retrieved endonasally, and looped.

Finally, the initial nasal flap was reflected back and cut to create an "L"-shaped flap, covering the posterior and inferior part of the new rhinostomy. We did not routinely use nasal packing after DCR surgery, except in two cases of intense post-operative bleeding. In these cases, packings were removed on the first post-operative day.

All patients received post-operative oral antibiotics (amoxicillin + clavulanate) and were instructed to perform nasal saline douching, use emollient local ointment, and apply antibiotic-steroid eye drops for a 7-day period. Local nasal therapy was continued until the rhinostomy was entirely healed.

Follow-up visits were performed on days 1 and 4, once a week for the first month, once a month for 3 months, every 6 months for 1 year and then once a year. Post-operative follow-up ranged from 4 to 168 months (mean = 38.07). All clinical evaluations consisted in lacrimal pathway irrigation and nasal endoscopy to evaluate patency of the rhinostomy.

The silicone Catalano's tube remained in place from 3 to 4 months. Anatomical success was described when a patent ostium on irrigation was achieved, whereas functional success was defined as free lacrimal flow on functional test and resolution of epiphora.

Results

The study group included 502 procedures using endoscopic endonasal powered DCR on 401 Caucasian patients (110 males and 291 females), aged 5–84 years (average age = 58 years), with a

diagnosis of NLDO. In our case series, we collected both primary and secondary NLDO: secondary causes included those due to facial trauma (n = 5), radioiodine therapy (n = 3), radiotherapy (n = 3), Wegener granulomatosis (n = 5), and chemotherapy (n = 1).

The male to female ratio was 1:2.6 (110:291); 92 of the 502 surgical procedures were presented in a previous publication.[1] During our study, four subjects were excluded because of diagnosis of malignancy (two melanomas, one squamous cell carcinoma, and one inverted papilloma). Of the 498 procedures, 426 were unilateral and 72 were bilateral DCRs.

Considering all END-DCRs, 80.7% were primary (402/498) and 19.3% (96/498) were secondary (Table 1). When primary END-DCR was not efficient in treatment of epiphora, we usually performed a second endoscopic procedure.

Table 1. Results of primary and secondary dacryocystorhinostomy.

	Primary DCRs	Secondary DCRs	Primary + secondary DCRs
Number of procedures	402	96	498
Anatomical success	91.5% (368)	89.4% (84)	90.1% (449)
Functional success	90.4% (362)	85.1% (80)	88.7% (442)

DCR: dacryocystorhinostomy.

Among revision cases, external DCR was previously performed in 43.75% (42/96) patients, an endoscopic approach in 37.5% (36/96), and transcanalicular surgery in 18.75% (18/96).

Only two patients who had received two END-DCRs from our institution underwent a third endoscopic procedure, with a success rate of 50%. In all patients with nonsuccessful surgical therapy after two DCRs and in one patient who received three END-DCRs at San Raffaele Hospital, a Jones tube was placed.

When END-DCR was performed, a Catalano's stent was placed for 3–4 months: in particular, only five patients kept the stent in place for 4 months due to personal issues. The mean operative time for primary DCR was 25 min (range = 15–35), while it was 22 min (range = 10–40) for secondary DCR.

All patients examined presented significant preoperative epiphora that was associated with purulent discharge (n = 58), acute dacryocystitis (16 in primary DCRs, 8 in secondary DCRs), dacryocystocele (n = 6),[11] and presence of lacrimal calculi (n = 2).

During END-DCR, 85 patients (21.2%) required the following as an additional procedure: 10.5% (42/401) endoscopic septoplasty,[12] 5.7% adjunctive sinonasal surgery, 2.7% middle turbinate plasty, 2% synechia lysis, 0.3% (n = 1) dental implantation treatment,[13] and 0.3% (n = 1) punctumplasty.

Observed complications included epistaxis (n = 4), edema of the eyelid (n = 25), turbino-septal synechia (n = 8), laceration of the lacrimal canaliculi (n = 3), ostium granulomas (n = 15; 6 treated with topical cortisone, 9 treated surgically),[14] and lacrimal stent dislocations (n = 16) that were properly repositioned with a nasal endoscope.

At last follow-up, final anatomic success was achieved in 91.54% cases in primary DCR and in 89.36% in revisions, whereas a functional result was obtained in 90.4% in primary and 85.1% in secondary DCRs. Considering the initial endoscopic procedure (498), DCR surgery was anatomically successful in 90.1% and functionally successful in 88.7% of procedures.

Among treated cases, no factors showed a significant difference between successful and unsuccessful treatment, including sex (p = 0.561) or age (p = 0.240). Only a history of chemotherapy (p = 0.001) and radiotherapy (p = 0.04) had significant p values; however, in our case series, there were few patients with such secondary NLDO causes (chemotherapy = 1, radiotherapy = 3), making the results of little statistical relevance.

No other causes of NLDO showed significant p values (p > 0.05). Statistical analysis was not performed for timing of silicone stenting or race due to the homogeneity of cases.

Discussion

NLDO is a common pathology that can be treated with various types of approaches, both surgical and nonsurgical. Nasal END-DCR is one of the most widely used techniques since it usually achieves high success rates with low morbidity and aesthetic problems.[10]

While the success rates of END-DCR in the literature range from 75% to 96%,[3,9,15–18] our retrospective case series documented an anatomic success rate of 91.54% in primary DCR and 89.36% in revisions, with a functional success of 90.4% in primary and 85.1% in secondary DCRs.

In only two patients were three END-DCRs needed, with a functional success rate of 50%. In particular, the patient who did not achieve a functional result had been previously treated for a thyroid cancer with I-131,

which is known to alter the healing process in 2.2%–18% of patients.[19]

To establish the correct approach to NLDO, prior to surgery, accurate and specific diagnosis was made through multidisciplinary consultation between an ophthalmologist and an otorhinolaryngologist. All patients should follow a diagnostic workup that starts with the Jones test 1 and 2 (fluorescein test), usually performed by an ophthalmologist. These tests are considered non-invasive procedures that determine if lacrimal stenosis is functional or obstructive. Probing and irrigation of the lacrimal system is the second ophthalmologic step and is a safe, easy, and low cost way to establish correct diagnosis. These clinical evaluations can be used, in the majority of cases, to diagnose nasolacrimal obstruction.[20] Some authors routinely complete diagnostic workup with dacryocystography or dacryoscintigraphy, which may be useful in detecting nasolacrimal anatomy. The review proposed by Lefebvre and Freitag[20] suggests that these radiological tools are useful only when there is suspicion of complicated anatomy or a need for re-operation.

In order to evaluate nasal anatomy, we usually perform nasal endoscopy and maxilla-facial CT scan, which are helpful in detecting the exact position of the uncinate process and its relationship with the lacrimal system. In addition to this, radiological imaging can reveal potential sinus diseases and pneumatization of the agger nasi. However, especially in the pediatric population, CT radiation can be an issue, but, in agreement with the review of Lefebvre and Freitag,[20] the additional significant information that can be obtained from a CT scan (sinonasal malformations or pathologies such as concomitant sinusitis or concha bullosa of the middle turbinate) is useful to perform a correct END-DCR.

To prevent early obliteration of the new rhinostomy after END-DCR, we used a Catalano's stent that was kept in place for 3–4 months. This silicone device may lead to correct healing, but can cause formation of granulation tissue, infection, and ulceration of the lacrimal pathway. In addition to this, it can dislocate and cause patient discomfort, leading to mandatory stent re-positioning.[1]

In the literature, different approaches to NLDO have been used with a wide range of success rates.[3,5,8,10,15–17,21–25] In particular, NLDO can be treated with both surgical techniques, such as external dacryocystorhinostomy (EXTDCR) and END-DCR, and nonsurgical procedures, such as radiological placement of nasolacrimal stents[24–27] and balloon dacryocystoplasty.[22,23] Radiological intervention has a significantly lower success rate, but can be used in specific types of patients who cannot be subjected to general anesthesia.[28] In recent years, many groups have compared EXT-DCR and END-DCR.[10,15,21,29–31] Huang et al.[10] carried out a meta-analysis and systematic review on surgical DCR approaches: comparable results were seen between EXT-DCR and mechanical END-DCR with reported revision surgeries similar in both approaches (risk ratio (RR) = 1.02; confidence interval (CI) = 0.98–1.06). Hartikainen et al.[9] compared endonasal laser-assisted DCR with external DCR and reported significantly better outcomes with EXT-DCR (63% vs 91%). However, ENDDCR has some advantages over EXT-DCR such as limited invasiveness, shorter operative time, preservation of pump function, less bleeding, and absence of skin trauma that can lead to an external scar.[1] Recently, Ng et al.[32] described a new surgical EXT-DCR technique in which the external cutaneous scar can be avoided, thanks to a periciliary incision, making the procedure more aesthetically desirable, with a functional success rate of 83.3%. Similar aesthetic external surgical approaches have been proposed by other authors with variable results.[33–37]

Compared to our previous study,[1] anatomical and functional success rates seem to be lower. This is possibly due to the greater number of procedures performed, heterogeneity of patients, specific multidisciplinary selection of patients, and longer follow-up time.

When END-DCR is concluded, some authors complete the procedure with silicone intubation of the nasolacrimal pathway.[38] Kim et al.[39] evaluated the effect of silicone stent intubation during END-DCRs by meta-analysis: it was reported that, even if there was no significant heterogeneity between the studies analyzed and the use of a silicone stent seems to increase the success rate compared to the control group (odds ratio (OR) = 1.45; 95% CI = 0.77– 2.73; p = 0.244), there was no statistically significant difference in outcomes. Indeed, the use of a silicone stent and its duration have not been definitively demonstrated to be effective.

Some authors suggested that the use of mitomycin C (MMC), an aminoglycoside antibiotic with antineoplastic potential, may improve the success rates of END-DCR,[40] whereas other authors suggested that it does not influence outcomes.[41–44] Local instillation of MMC has no systemic side effects, but can cause conjunctival irritation, lacrimation, and punctate keratitis.[45]

Cheng et al. published a systematic review and metaanalysis reporting that MMC improves success rates in primary and revision END-DCR without silicone stenting, but no differences were noted in the subgroup of silicone intubation. For these reasons, we do not apply MMC during END-DCR.

Finally, many authors have suggested that correct timing for follow-up is fundamental as it can radically influence the surgical result:[3,6] in fact,

periodic lacrimal pathway irrigation and scar tissue removal is an important aspect of postoperative follow-up. Moreover, some authors suggest that endoscopic nasal toilette, using a 30° rigid endoscope, is a possible clinical technique to obtain a functional rhinostomy after END-DCR because it allows removal of nasal granulation tissue, scars, and pathological secretions.[3]

In conclusion, this retrospective case series confirms that END-DCR is one of the most successful types of surgery in treatment of NLDO. It is important to emphasize that meticulous endoscopic surgery and precise follow-up are key factors in obtaining anatomical and functional patency of the nasal rhinostomy in the long term.

Declaration of conflicting interests

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Influence of Surgical Techniques on Endoscopic Dacryocystorhinostomy: A Systematic Review and Meta-analysis

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Abstract

Objective. Endoscopic endonasal dacryocystorhinostomy (ENDDCR) has increased as a valid alternative to the classic external approach to treat distal lacrimal obstruction. Different surgical varieties of the END-DCR approach have been proposed with no clear understanding of the best surgical technique.

Data Source. A comprehensive research was performed in PubMed, Embase, SCOPUS, and Cochrane databases with a final search on March 2020.

Review Methods. The aim of this search was to identify relevant END-DCR procedures performed with mechanical (MecnEND-DCR) and powered (Pow-END-DCR) approaches to compare their functional success rate. In addition, the influence of mucosal flaps was evaluated. Articles were selected only if they were published later than 2000 and had at least 50 single-clinician surgical procedures performed. Excluded articles included acute infections, cancers, mixed cohort study, and revision cases.

Results. A total of 11,445 publications were identified and 2741 reviewed after screening; 15 articles were included after full-text review (0.6% of the initial articles reviewed). The mean success rate was 91.34% (95% CI, 87.1%-94.3%) for Pow-END-DCR and 89.5% (95% CI, 86.5%-91.9%) for Mecn-END-DCR with no significant difference between the surgical approaches (P = .43). For mucosal flaps performed during END-DCR, the mean success rate was 89% (95% CI, 86%-91%) if mucosal flaps were used and 92% (95% CI, 88%-95%) if they were not used, with no statistical difference present (P = .14).

Conclusions. Our analyses suggest that there are no differences in outcomes between mechanical and powered approaches in END-DCR and that mucosal flap preservation is not essential to achieve a superior END-DCR outcome.

Keywords systematic review, dacryocystorhinostomy, DCR, mucosal flap, powered, mechanical

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Lacrimal pathway obstruction is one of the most common causes of epiphora, a clinical sign that can be present in other pathologies, such as lacrimal pump failure and lacrimal hypersecretion.[1] Obstructive lacrimal pathologies can be divided into acquired and congenital forms and, in relation to the site of the obstruction, into proximal and distal. Epidemiologically, distal acquired lacrimal obstruction (DALO) is the most common form and can be caused by different disorders[2-6]; in absence of other factors, stenosis is known as idiopathic.[1] In the past, the gold standard treatment of DALO was external dacryocystorhinostomy (EXT-DCR) since the success rates were superior than other procedures, as it seemed that with open access, the surgeon was able to achieve complete exposure of the lacrimal sac with a larger and more stable neorhinostomy.[7]

At the beginning of this century, in addition to the technological revolution of endoscopic endonasal surgery, a lacrimal anatomic study that thoroughly influenced subsequent endoscopic approaches was performed: in fact, Wormald et al underlined that the major portion of the lacrimal sac lies above the axilla of the middle turbinate rather than anteroinferiorly.[8] This knowledge permitted development of a new endoscopic endonasal dacryocystorhinostomy (ENDDCR) technique that imitated the external approach by allowing exposure of the entire lacrimal sac, creating the largest possible dimension of the neorhinostomy.[9-11] However, in the majority of cases, the bone of the lateral nasal wall became progressively thicker as the dissection proceeds up to the axilla of the middle turbinate so that the

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cold instruments usually applied in endoscopic mechanical END-DCR (Mecn-END-DCR) did not seem to be appropriate.[9] To resolve this problem, a powered approach (PowEND-DCR) has been described that allowed for superior bone removal through the application of different steel burrs.[7] Nevertheless, given these endoscopic approaches, increasing concerns arose about the risk of mucosal scar formation, the high rate of granulation tissue, and synechiae formation; accordingly, different mucosal flaps were reported to increase the success rate of the surgical procedure.[12]

Nowadays, END-DCR seems to have comparable results to EXT-DCR,[13,14] although, at present, no study has compared the mechanical and powered approaches on END-DCR outcomes, and no consensus has been achieved on the benefit of mucosal flaps. The aim of this article is to systematically review the available literature on the influence of the surgical technique and mucosal flaps in END-DCR procedures and to investigate the success rates of these different approaches.

Methods Search Methods for the Identification of Studies

PubMed, EMBASE, Scopus, and the Cochrane Library underwent a structured search in October 2019 to identify all studies related to this review; the following search terms were used: ((dcr[title]) OR dacryocystorhinostomy). The systematic review was updated in March 2020 and was performed in accordance with the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-analyses; Supplemental Table S1, available online).[15]

The initial selection was based on the title and abstract of the article, whereas a second selection was based on full-text review. Additional studies were identified by hand-searching the reference sections of articles reviewed. Selection of publications was done by searching articles in the English language that satisfied the following conditions: the study (1) evaluated patients with DALO; (2) included an abstract; (3) was published later than 2000; (4) indicated a clear type of surgery performed; (5) comprised only primary cases; (6) had a clear functional success value; and (7) was based on .50 single-clinician procedures. Regarding the last point, as highlighted in recent literature, the lacrimal surgical learning curve can influence outcomes so that an experienced clinician may diminish a potential bias.[16-18] Exclusion criteria were as follows: the study (1) comprised a mixed cohort of congenital and acquired etiology, (2) analyzed surgical success only in secondary cases, (3) did not clearly define the surgical success rate, (4) included oncologic pathologies, or (5) was primarily based on surgery performed for acute infections (dacryocystitis).

Case reports, editorials, letters, ''how I do it'' articles, descriptions, and reviews were excluded.

Data Extraction

From each article, the following information was independently extracted by 2 authors (A.V. and M.T.): surgical procedure, publication data, first author, number of events, mean age, mean follow-up, article type according to the Oxford Centre for Evidence-Based Medicine,[19-22] type of procedure performed, mean success rate, use of mucosal flaps, and other potential bias. The data extracted by the 2 authors were compared, and any disagreement was resolved by discussion to reach consensus.

Quality Assessment

The type of article was evaluated according to the guidelines in chapter 8 of the Cochrane Handbook for Systematic
Reviews of Interventions.[23]

In terms of study quality, assessment was performed independently by 2 investigators using MINORS (Methodological Index for Nonrandomized Studies), which is a validated tool designed for nonrandomized controlled trials. This instrument is based on 8 items for noncomparative studies and 12 items for comparative studies, each of which has a score of 0 (not reported), 1 (reported but inadequate), or 2 (reported and adequate).[24] However, given the inclusion of 2 randomized controlled trials, its risk of bias was assessed with the revised Cochrane risk-of-bias tool (Table 1).

Outcome Measures

The principal outcome considered was functional success rate, defined as a score \2 on the Munk scale[25] or complete resolution/significant improvement of tearing; in addition, a minimum follow-up of 3 months was required.

The studies included had to describe an END-DCR surgical procedure that could be performed in 2 ways: mechanically (Mecn-END-DCR), which means that only cold instruments were used for bone removal, or powered (PowEND-DCR), which includes diamond burr usage in bone removal, particularly in the upper portion of lateral nasal wall. The outcomes of surgery, based on functional success rates, and additional mucosal flaps harvested were compared.

Statistical Analysis

All the statistical analyses were performed with R (version 3.6.2),[26] and for the quantitative analyses of mean success rate (meta-analysis), the meta package was selected.[27] The I^2 statistic was used to measure heterogeneity among articles. When this value highlighted significant heterogeneity, a random effects model was considered to evaluate the success rate of the techniques.

Due to the low sample size, comparison of age, mean follow-up time, and number of procedures in different surgical procedures was tested by means of a Mann-Whitney test. Statistical significance was considered for P values \.05.

Risk of Bias

Our primary outcome was dichotomous (ie, ‘‘Did the type of END-DCR surgical approach/mucosal flaps influence the surgical outcome?’’), reducing the risk of reporting bias. Anatomic success rate was studied but not reported.

END-DCRYes
END-DCRNo
END-DCRYesIncludedprecedentocular

Table1. CharacteristicsofIncludedStudies.

FirstAuthorYear	Typeof article [a]	Studyquality [b]	No.of procedures	Meandacryocystorhinostomy success rate,%	Mean follow-up,mo	Typeofsurgery	Mucosal flaps	Additionalbias

Highriskofbias8190.1012Mecn-END-DCRNo
Highriskofbias9296.7012Mecn-END-DCRNo
END-DCRYesNasoporepacking
END-DCRYesMerocelpacking
END-DCRNoHeterogeneityinstenttiming
Al-Qahtani[28]
END-DCRYesNasoporepacking
Ayoob[29]
END-DCRYesNasoporepacking
Beshay[30]
END-DCRNoGuardix-Solpacking,
Ciğer[31]
Do[32]
END-DCRNoHyFencepacking,heterogeneity
20164628881.303.2Mecn-
20184712092.5046.5Pow-END-
END-DCRNoMediclorepacking, mitomycinC
20134750926
20164133190.306Mecn-
ErfanianSalim[33]
END-DCRYesNasoporepacking
Karim[34]
END-DCRYesIncluded8revisioncases
DCRYes5caseswithPinostent
20164135583.606Mecn-
trauma,heterogeneityinstent timing
20154712895.306Pow-
Lee[35]
20164137494.606Mecn-
Mak[36]
Park[37]
1 sodium hyaluronate
201141310582.406Mecn-
Shin[38]
201541331889.303Mecn-
heterogeneityinstenttiming
201541326275.903Mecn-
instenttiming
201741210593.303Mecn-
2013477993.7023Mecn-
heterogeneityinstenttiming
Highriskofbias11294.609Pow-END-DCRYesMerogelpacking
Su[39]
201741214985.203Mecn-
Highriskofbias115809Pow-END-DCRYes
Trimarchi[40]
20184137191.506Mecn-
Trimarchi[41]
Wu[42]

20184138991.10892.106Mecn-
Abbreviations:Mecn-END-DCR,mechanicalendonasaldacryocystorhinostomy;Pow-END-DCR,poweredendonasaldacryocystorhinotomy.
[a]LevelofevidenceaccordingtotheOxfordCenterforEvidence-BasedMedicine.
20184121079.706Mecn-
[b]StudyqualityfornonrandomizedstudiesisbasedonamaximumscoreofI6fornoncomparativestudiesand24forcomparativestudiespertheMINORScriteria(MethodologicalIndexforNonrandomized Studies).StudyqualityforrandomizedcontrolledtrialsisassessedbytherevisedCochranerisk-of-biastoolforrandomizedtrials.

20094194194380747038.07Pow-END-

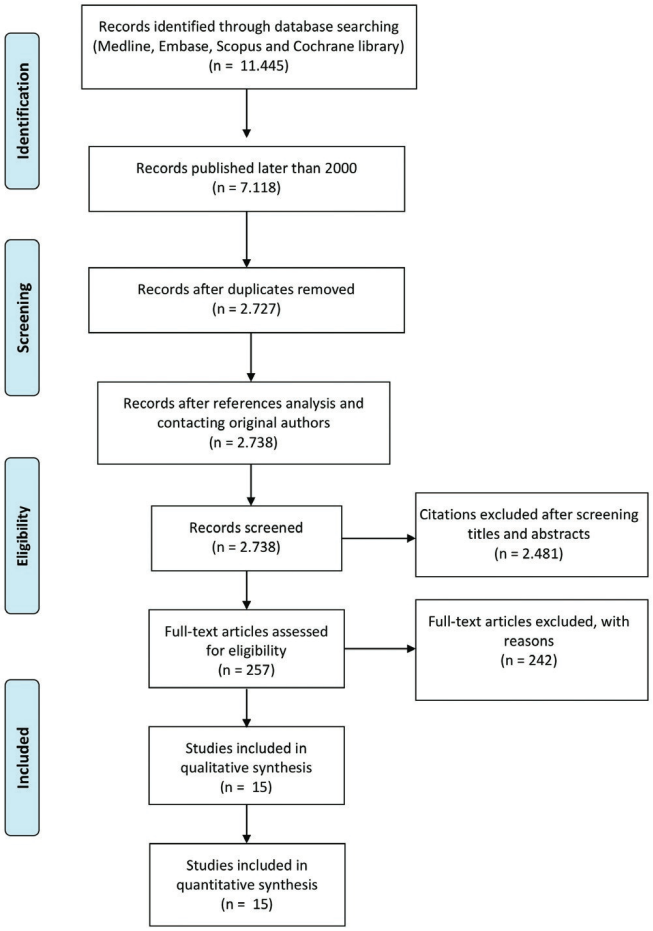

Figure 1. PRISMA flow diagram for the method of data collection and the selection of studies.

Results

From PubMed (Medline), Embase, SCOPUS, and the Cochrane Library, a total of 11,445 articles were initially retrieved. When search limits were fitted and duplicates removed, 2727 were selected; in addition, 10 records were found in the reference lists of the articles reviewed, and 1 corresponding author was successfully contacted. On the basis of their titles and abstracts, 2481 articles were considered ineligible, principally because of the following: a mixed cohort of surgical procedures was performed; the study examined congenital and acquired pathologies; it did not define a clear functional success rate; the procedures were not based on a single clinician; and the study included neoplastic and infective forms of distal lacrimal obstruction. Based on review of the plain text, 15 studies were ultimately selected (Figure 1).[28-42] According to the Oxford Centre for Evidence-Based Medicine,[21] 13 articles had an evidence level of 4, and 2 articles had an evidence level of 2. All included articles were published later than 2000 and provided a clear functional success rate based on singleclinician procedures; in fact, as recently demonstrated, an experienced surgeon can positively influence the ENDDCR outcome.[16-18]

Due to the lack of reliable studies, only articles that analyzed EXT-DCR and END-DCR were included if the endoscopic procedures satisfied the inclusion criteria; however, no analyses were done on the EXT-DCR procedure (Table 1).

In total, 5 articles described a Pow-END-DCR approach, whereas 10 described Mecn-END-DCR. As a consequence, 3059 procedures were selected from 15 articles: 1065 (34.8%) Pow-END-DCR and 1994 (65.2%) Mecn-END-DCR.

For these 2 END-DCR surgical approaches, no significant difference was noted for the number of procedures of each study (P = .06), whereas a difference was noted in mean follow-up time (P = .01) and mean age (P = .046), which could be caused by the lower number of Pow-END-DCR studies.

The mean success rate heterogeneity among studies was high (I^2 = 74%); thus, a random effects model was fitted. Mean success rate model estimates are 91.3% (95% CI,
87.1%-94.3%) for Pow-END-DCR and 89.5% (95% CI, 86.5%-91.9%) for Mecn-END-DCR; however, no significant difference was observed between these approaches (P = .43; Figure 2).

For use of mucosal flaps during END-DCR (mechanical and powered), 9 of 15 studies described the method of harvest: 6 Mecn-END-DCR and 3 Pow-END-DCR. There was a predominance of posterior-based (3/9) and lacrimal sac (3/9) flaps, followed by H-shape flaps (2/9); in 1 case, a general mucosal flap was described with no technical specifications (Table 1). The random effect model (I^2 = 74%) mean success rate estimate was 89% (95% CI, 86%-91%) in case of mucosal flap usage and 92% (95% CI, 88%-95%) if not used. The difference in success rate was not significant (P = .14; Figure 3).

The influence of mitomycin C (MMC) on the surgical outcome was not feasible due to the heterogeneous timing, concentration, and modality of MMC application.

In total, 12 of 15 articles (80%) described the application of endocanalicular stents (15 Mecn-END-DCR and 3 PowEND-DCR), both mono- and bicanalicular. The average duration before stent removal ranged from 1 to 6 months. Regarding the influence of these silicone devices on the surgical outcome of Mecn-END-DCR and Pow-END-DCR, no quantitative analyses were feasible due to the lack of data.

The data that support the findings of this study are available from the corresponding author upon request.

Discussion

END-DCR has been recently considered, in association with EXT-DCR, to be one of the main surgical approaches in the treatment of DALO.[14] In the literature, there are technical varieties of END-DCR, such as mechanical versus powered approaches and mucosal flap versus no mucosal flap. However, in the last 15 years, these approaches have never been compared. In fact, to the best of our knowledge, the present analysis represents the first attempt to compare these challenge arguments.

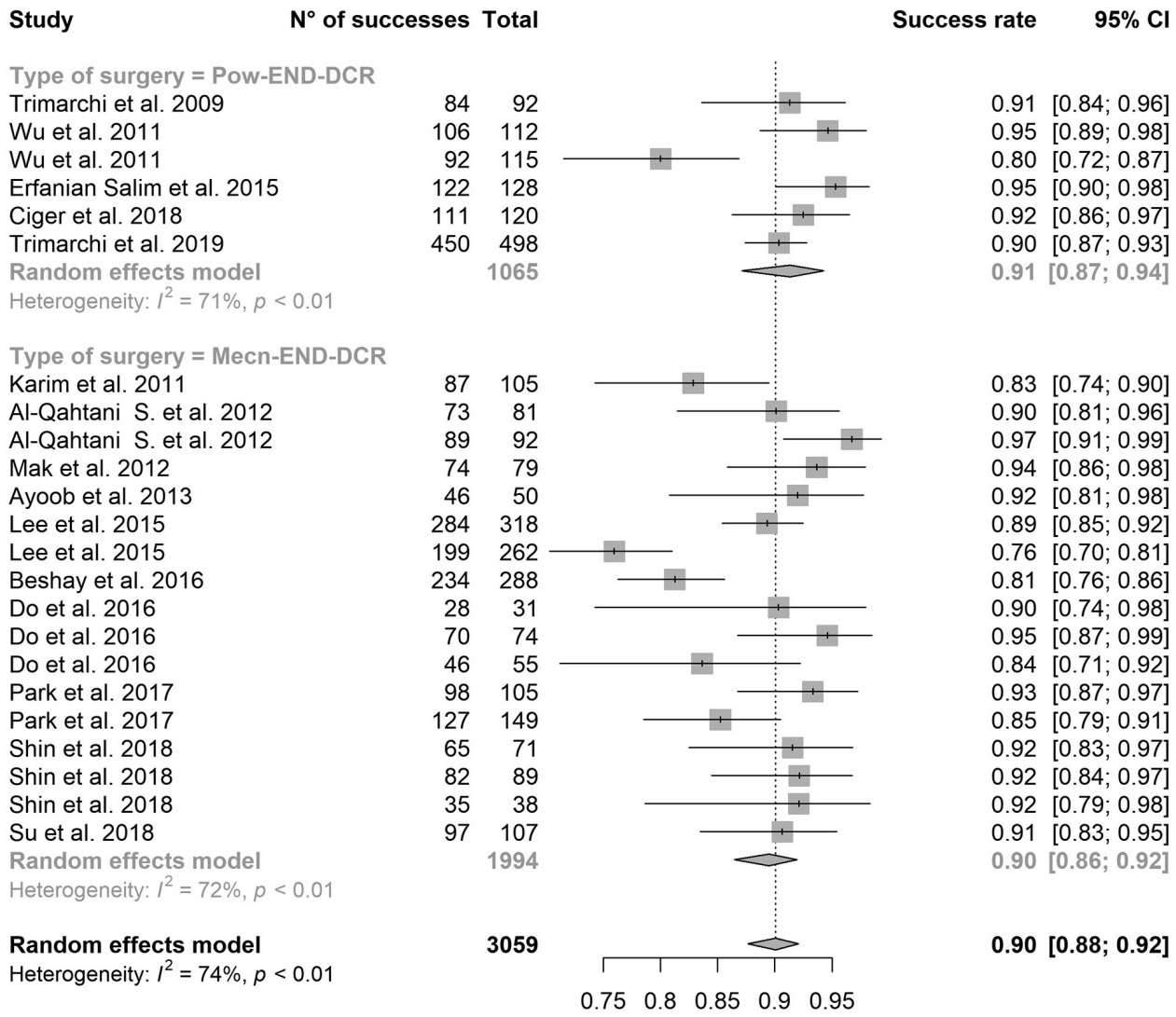

Figure 2. Forest plot for the subgroup analysis of Pow-END-DCR and Mecn-END-DCR. Success rates are presented with corresponding 95% CIs for all studies. Diamonds represent the group estimate from the random effects model. Mecn-END-DCR, mechanic endonasal dacryocystorhinostomy; Pow-END-DCR, powered endonasal dacryocystorhinostomy.

Pow-END-DCR is a technical variety of END-DCR that has gained relevance due to recent anatomic considerations.[7,11] In fact, given the position of the lacrimal sac and the thickness of the upper lateral nasal wall, a powered approach has been held to be useful to achieve the largest bony rhinostomy with complete exposition of the lacrimal sac.[9] As already stated by others, contracture of the ostium size is known to occur during the healing process and ranges between 50% and 92% of the original diameter; hence, the bigger the neorhinostomy, the better the outcome should be.[42,43] However, no direct correlation has been demonstrated between the initial neorhinostomy diameter and the final functional success rate.[12,44]

For our data, although there is no significant difference between Mecn- and Pow-END-DCR (P = .43), the majority of investigations (10/15) described a mechanical approach, with 65% of the total procedures performed with Mecn-END-DCR.

This evidence originates from previously described issues. First, it has been suggested that Mecn-END-DCR provides a faster surgical approach.[45,46] Second, powered instruments may produce mechanical and thermal injuries to the nasal mucosa due to the rotating burr, which in addition to this, may also produce bone dust. This biological substance can deposit onto the surgical field causing irritation, granulation tissue, and difficult visualization, particularly under local anaesthesia.[47] Moreover, some authors claimed that the powered approach does not provide a larger rhinostomy as compared with that created with mechanical instruments.[48] However, other authors have claimed that powered approaches are faster and associated with better outcomes when compared with mechanical ones.[7,44]

Given all these open issues, the role of adjunctive procedures that may influence the surgical outcome of END-DCR warrants comment. A common reason for the failure of this surgical approach is late rhinostomy closure caused by granulation tissue and synechiae such that, in the past, several surgical variations have been proposed, with attention to mucosal flaps.[12] As documented by many authors,[12,49] less bone exposure provides a better healing process and less formation of granulation tissue, which, in the long term, guarantees better outcomes, similar to widely accepted arguments for sinus surgery.[50-52] Nevertheless, comparable outcomes in case series treated with no mucosal flaps have been reported,[44,53-56] making this possibility controversial. Herein, we found reporting on harvesting of a mucosal flap (either nasal mucosa and lacrimal sac mucosa) in the majority of publications (9/15), and no significant differences were noted (P = .14). As a consequence, this evidence underlines that second-intention mucosal healing can provide reliable clinical outcomes. However, as recently highlighted, it may be associated with greater risk for formation of granulation tissue and mucosal contraction, which may be avoided with meticulous surgical technique and minimal degree of bone exposure.[44] Additionally, in case of mucosal sacrifice, it seems that more endoscopic debridements are needed to achieve a good result.[57] Due to the high technical varieties of mucosal flaps (U-shape, L-shape, free nasal mucosal flap, and lacrimal sac flap), statistical analysis on the precise techniques performed was not feasible.

Given the available adjunctive therapies that may improve success rates, many treatments have been tried

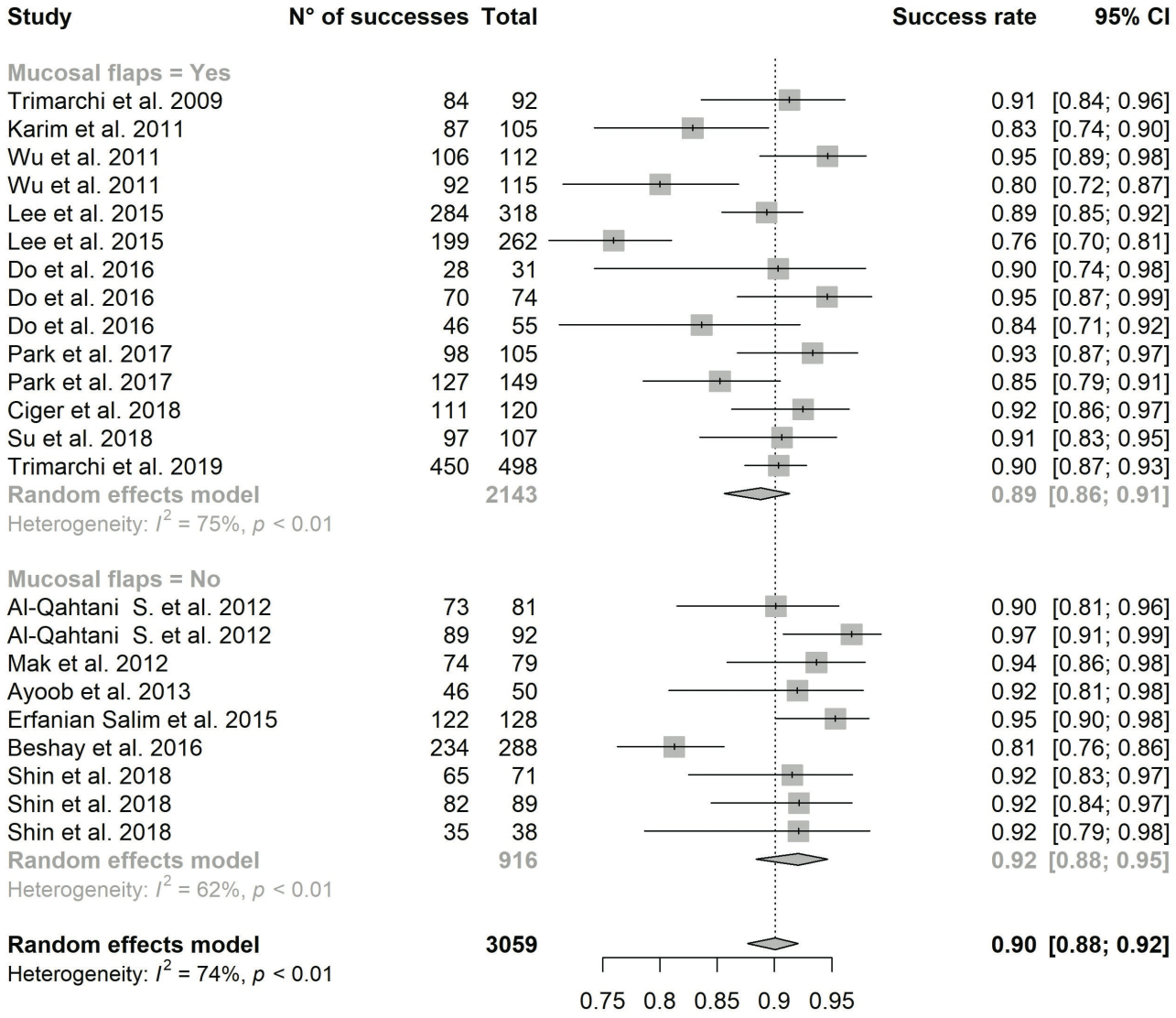

Figure 3. Forest plot comparing END-DCR performed with and without mucosal flaps. Success rates are presented with corresponding
95% CIs for all studies. Diamonds represent the group estimate from the random effects model. END-DCR, endoscopic dacryocystorhinostomy.

over the last decades with attention to application of silicone stenting and local MMC. If bicanalicular stents seem to provide better results, even the differences are not significant as compared with the control group,[58-62] As an antineoplastic agent that prevent fibroblast proliferation, MMC can be used in a variety of modalities, timing, and concentration, making its influence unclear and usage not standardized.[14] In fact, as stated by many authors, the function of MMC has not been fully understood, and its role must still be determined.[63-66]

This analysis has some limitations. First, even if 3 months of follow-up has been considered to be a stable timing to evaluate the neorhinostomy, it cannot be excluded as a potential bias. Second, the pooled results are of different levels of evidence due to the limited number of studies. Third, the heterogeneity of functional success measurement (symptoms questionnaire, visual analog scale, or reported symptoms relief), which was considered the main surgical outcome, has to be included as an additional potential bias. Fourth, all articles treated acquired lacrimal obstruction, which can occur in childhood and adulthood; thus, an age difference must be considered a source of heterogeneity. Fifth, the demonstrated absence of significant differences in surgical techniques and mucosal flaps usage could have been influenced by the relative lack of reliable data (doubleblinded randomized trial), leading to a potential fail detection of real existing differences.

In conclusion, END-DCR is now considered one of the most successful surgical approaches to treat DALO, but given the increasing relevance of such a procedure, technical varieties have been proposed with no demonstrated superiority of one over another. In particular, mechanical and powered END-DCR approaches provide comparable results, and mucosal flap preservation is not essential to achieve superior outcomes. Given the high success rates of END-DCR and a nonunanimous consensus on the real influence of these techniques, large randomized trials are clearly needed.

Author Contributions

Alessandro Vinciguerra, made substantial contributions to conception, design and acquisition of data, drafted the article and revised it, gave final approval of the version, and agreed to be accountable for all aspects of the work; Alessandro Nonis, made substantial contributions to the analysis and interpretation of data, drafted the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work; Antonio Giordano Resti, made substantial contributions to conception of the data, revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work; Diego Barbieri, made substantial contributions to conception of the data, revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work; Mario Bussi, made substantial contributions to conception of the data, revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; Matteo Trimarchi, made substantial contributions to conception of the data, revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosures

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Supplemental Material

Additional supporting information is available in the online version of the article.

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SHORT COMMUNICATION

Acetic acid disinfection as a potential adjunctive therapy for non-severe COVID-19

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Abstract

Purpose SARS-CoV-2 is a new pandemic influenza caused by a coronavirus which main route of transmission is through exhaled droplets that primarily infect the nose and the nasopharynx. The aim of this paper is to evaluate the effect of acetic acid, the active component of vinegar, as a potential disinfectant agent for upper airways.

Methods Twenty-nine patients were enrolled and divided into two groups: group 1 (14 patients) was composed of patients treated with off-label hydroxychloroquine and lopinavir/ritonavir, whereas group 2 (15 patients) was composed of patients treated with hydroxychloroquine only, combined with the inhalation of acetic acid disinfectant at a 0.34% concentration. A questionnaire-based evaluation of symptoms was performed after 15 days in both groups.

Results It appears that the number of patients treated with acetic acid (group 2) that experienced improvement in individual symptoms was double that of the other group of patients (group 1), although numbers are too small for robust statistical analysis.

Conclusions Considering its potential benefits and high availability, acetic acid disinfection appears to be a promising adjunctive therapy in cases of non-severe COVID-19 and deserves further investigation.

Keywords COVID-19 · Acid acetic · Disinfection · Coronavirus

Introduction

The main route of transmission of the new coronavirus called COVID-19 (SARS-CoV-2) is through mucosal contact with infected exhaled droplets [1]. Considering the higher viral load detected in the nose, compared with the lower respiratory tract [2], this may be one of the first anatomical structures exposed to viral contagion as evidenced by the fact that acute anosmia is a relatively common presenting symptom [3, 4]. With the rapid spread of COVID-19, global health-care systems have faced the challenge of treating an overwhelming number of patients for which there is no widespread immunity. As a result, many off-label therapies have been tried but so far with uncertain results. In addition, the mild symptoms in the majority of cases and the saturation of hospital beds may compel doctors to treat the majority of infected people at home. In this situation, experimental treatments are not readily available to those at home or be available in low-resource setting [5].

Acetic acid, the active component of vinegar, is a commonly available disinfecting agent. Inhalation of a waterbased acetic acid solution to treat the symptoms of the common cold is a common folk remedy in Italy. Indeed, the anti-bacterial and anti-viral activities of acetic acid are documented in the literature [6–9]. Acetic acid causes inactivation and dis-aggregation of haemagglutinin glycoproteins (found on the surface of influenza viruses) by generating a low pH-dependent conformational change of those glycoproteins and it destroys the viral envelope and inhibits viral transmission [6]. Therefore, considering these antiviral properties and that the upper airways are the main site of SARS-CoV-2 entry and replication, we have explored the use of this historical therapy in early-stage cases of COVID19 by evaluating patients who were administered a disinfecting formulation of acetic acid as an intranasal aerosol.

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Materials and methods

In this prospective study, we included patients attending the Emergency Department of Cremona Hospital with positive real-time polymerase chain reaction (RT-PCR) for SARSCoV-2 performed on nasopharyngeal swabs.

The study was conducted according to the ethical standards established in the 1964 Declaration of Helsinki (revised in 2013) and was approved by the local ethics committee (Comitato etico Val Padana, Protocol No. 63-2020-OSS_ALTRO-CR45).

Records of patients positive for COVID-19 infection with mild symptoms who were discharged home after accessing the Emergency Room over March 2020 were evaluated and included in the study. Exclusion criteria were asthma (due to the possible bronchoconstriction from the acetic fumes) and intolerance to sulphites.

Patients treated with off-label hydroxychloroquine and lopinavir/ritonavir, the standard treatment prescribed at that time, were included in group 1, whereas group 2 was composed of patients treated with hydroxychloroquine only, combined with acetic acid nasal disinfectant at a 0.34% concentration.

While group 2 was created prospectively, group 1 was composed of patients that were recorded in the same period but retrospectively included in the study.

The solution was prepared by patients at home, using 3.5 dessert spoons (approximately 35 ml in total) of vinegar (with a 6% concentration of acetic acid) in 500 ml of boiling water. Patients were instructed to exclusively inhale the fumes of the solution through the nose using a commercially available device (Fig. 1) for 10 min twice a day. Inhalations could also be performed using a bowl with a towel over the head, with the only precaution of covering eyes to avoid possible local irritation.

Patients' symptoms at presentation, clinical course, symptoms after 15 days and the presence of SARS-CoV-2 on nasopharyngeal swabs taken after 15 days were recorded. Symptoms were evaluated through a questionnaire based on general questions on comorbidities, general and ENT symptoms associated with COVID-19 infection. The questionnaire only offered a binary answer (yes/no) to the following questions: Do you have/experienced any

improvement/ resolution of Cough? Fever? Shortness of breath? Vomiting/ Diarrhoea? Fatigue during the day? Headache? Nasal sneezing? Blockage/Congestion of the nose? Loss of smell/taste?

Table 1 Patient's questionnaire responses before (pre) and after (post) the therapy

	Group 1 pre *N* = 14	Group 1 post	Group 2 pre *N* = 15	Group 2 post
Cough	11	1	12	0
Fever	13	2	8	0
Dyspnoea	3	2	2	0
Vomit/diarrhoea	1	0	1	0
Fatigue during the day	6	5	6	3
Headache	3	2	4	0
Blockage/congestion of the nose	7	3	7	2
Anosmia	4	2	5	2
Dysgeusia	2	2	2	1

Group 1 control group, *Group 2* vinegar group

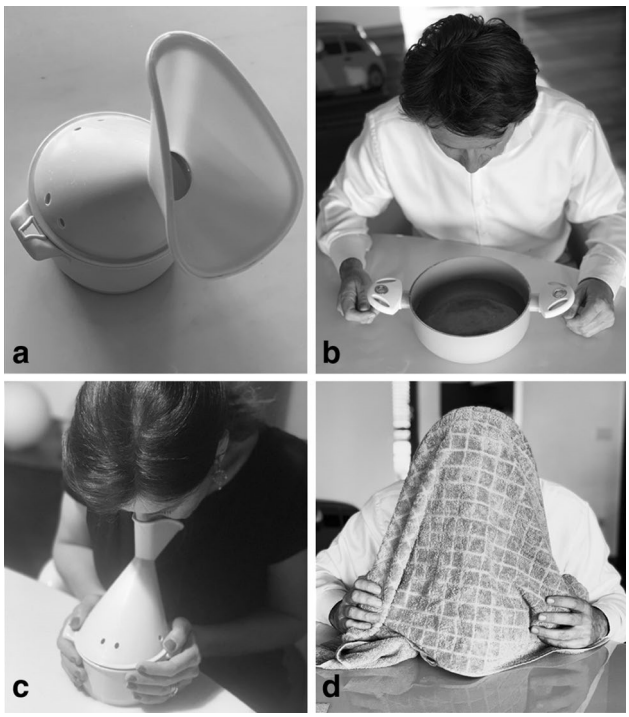

Fig. 1 An inhalation plastic device is shown (**a**). The mask is applied to the face to inhale the disinfectant solution (**c**). The acetic acid solution can be poured in a bowl (**b**) and fumes are inhaled placing the face above the bowl; the head is covered by a cloth to reduce fume dispersion (**d**)

Results

Twenty-nine patients were enrolled. There were 14 patients in group 1 (standard treatment) (7 females and 7 males, aged 33–73 years), and 15 patients in group 2 (acetic acid treatment) (11 males and 4 females, aged 49–80 years). Treatment compliance was 100% in both groups; no adverse events related to acetic acid inhalation were reported.

In group 1, all patients presented cough and fever, whereas in group 2, seven patients reported mild cold symptoms and eight patients cough and fever. All patients were followed for 15 days. In group 1, one patient was hospitalized because of increasing severity of respiratory symptoms, while 13 patients reported improvement of symptoms after 15 days. Seven patients out of 13 had a negative RT-PCR for COVID-19 at 15 days.

In group 2, after 15 days, all patients reported symptom improvement, 12/15 patients tested negative for SARSCoV-2, whereas 3/5 patients were still positive. Twice the number of patients experienced improvement in individual symptoms in group 2 than group 1 though numbers are too small for statistical analysis. (Table 1).

In all cases, the acetic acid inhalation was well tolerated as evidenced by compliance, although this was self-reported.

Discussion

The results of the study suggest a possible effect of acetic acid on the upper respiratory airway, which may lead to a faster clinical resolution of COVID-19. Our study is one of the first attempts to identify a topical therapy that could influence the natural history of COVID-19. The efficacy of acetic acid could be attributed to the exposure of respiratory mucosa, a known hotspot of viral replication, to a highly effective disinfectant. Acetic acid disinfection could not only improve the clinical course of COVID-19, but also reduce viral load in the airway and in the droplets exhaled by infected individuals. Thus, a possible beneficial effect of this topical treatment in reducing the spread of the disease can also be hypothesized.

The decision to avoid anti-viral therapies in group 2 was based on the increasing concern about the real benefits of that treatment and in the updated Italian guidelines (March 2020) [10], ritonavir/lopinavir is now

not considered to be effective. Therefore, taking into account the ineffectiveness of ritonavir/lopinavir, the pharmacological treatment regimens of the two groups can be considered equivalent.

This study have (has) some limitations: first, it would have been important to have a visual analogue scale to evaluate symptoms referred by the patients; second, some factors like age and gender differences between studied group could be considered a bias in the outcome of the study; third, due to the emergent situation, no anosmia testing was performed in the Emergency Department so that it is only based on self-reported symptom.

In conclusion, considering its potential benefits, high availability and absence of reported side effects, acetic acid disinfection appears to be a promising, cheap, easily administered and well-tolerated adjunctive therapy in cases of nonsevere COVID-19.

This is a small study performed at the height of the COVID crisis in a rapidly changing clinical environment. Additional studies on larger samples with a control inhalation arm are needed to provide high-quality evidence to further support the employment of this ancient remedy.

Author contributions LP and MT made substantial contribution to the concept and design of the work; GB and RM contributed to the data acquisition and its analysis; AV took his part in the interpretation of data and in drafting the article; VJL participated in revising the article critically and gave the final approval of the version to be submitted.

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Compliance with ethical standards

Conflict of interest None of the authors has any conflict of interest.

Ethical approval Approval was obtained from the ethics committee of Val Padana (No. 63-2020-OSS_ALTRO-CR45). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Informed consent Informed consent was obtained from each patient for treatment and use of de-identified clinical data for study purposes.

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Review

Otorhinolaryngological manifestations in granulomatosis with polyangiitis (Wegener's)

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article info

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Keywords: inflammation and vasculitis. Most of the initial symptoms begin in the head and neck region with a wide Cocaine spectrum of involvement of any site ranging from the nasal septum, paranasal sinuses, oral mucosa, larynx Crusting and even the external, middle and internal ear. Diagnosis may be delayed because the onset is heterogeneous
Otitis media and sometimes limited to one organ. The pathologic findings of a characteristic inflammatory reaction pat-
Fungal sinusitis tern, and the serum findings of elevated antineutrophil cytoplasmic antibodies can help to establish the diag-
Lymphoma

abstract

Granulomatosis with polyangiitis (Wegener's, GPA) is an uncommon disease of unknown etiology classically involves the ELK triad of the ear, nose, throat (E), lungs (L) and kidneys (K) with necrotizing granulomatous

Septal perforation nosis. The differentiation from other conditions that mimic GPA such as lymphoma and infections is of critical Subglottic stenosis importance to initiate appropriate treatment. Treatment of the underlying disease is medical with the use of Sarcoidosis immunosuppressive agents and will not be reviewed here. This review focuses on the otorhinolaryngologic Wegener's granulomatosis manifestation and complication of GPA as well as their surgical management and specifies the role of the ANCA otorhinolaryngologist as an integral member of the multidisciplinary care team for patients with GPA.
Granulomatosis with polyangiitis

Contents

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1. Introduction

Granulomatosis with polyangiitis (Wegener's, GPA) is an autoimmune disorder of unknown etiology characterized by necrotizing granulomatous inflammation and vasculitis that affects predominantly small vessels [1,2]. Larger vessels are rarely affected [3]. The disease has a predilection for the upper respiratory tract, lungs and kidneys, but any organ may be affected. GPA may remain limited to specific regions, such as the

nose and paranasal sinuses, or cause inflammation in various organ systems leading to multiple organ damage and failure [4]. The majority of patients with GPA have circulating anti-neutrophil cytoplasmic autoantibodies (ANCA) [5,6].

The mean survival of untreated generalized GPA is 5 months, and immunosuppressive therapy has improved the prognosis so that it is now a treatable, chronically relapsing disease with a median survival of 21.7 year after diagnosis [4,7].

The approach to care for patients with GPA is interdisciplinary, and firmly places the otorhinolaryngologist on the management team as upper respiratory tract involvement occurs in most patients at some stage in the course of disease [3,4].

When GPA is limited to the upper respiratory tract the differential diagnosis includes infections (spirochetes [syphilis, yaws], mycobacteria [tuberculosis, leprosy], bacteria [rhinoscleroma], fungus [aspergillus]) and other inflammatory conditions (sarcoidosis, Churg–Strauss syndrome, cocaine induced midline destructive lesions) [8,9]. Many of these lesions present with non-specific sinonasal symptoms and may progress rapidly to involve adjacent structures, such as the orbit and skull base, with significant clinical implications for timely diagnosis and management. Thorough diagnostic workup, including endoscopic, radiologic, histopathologic and serologic testing is imperative to arrive at a proper diagnosis and to initiate appropriate local and systemic treatment [10].

2. Nose and paranasal sinuses

Sinonasal involvement is the most frequent manifestation of GPA as it occurs in 85% of patients [11,12]. Nasal obstruction is often the first symptom, and hyposmia or anosmia are frequently experienced when extensive involvement of the nasal mucosa with mucosal swelling is present (Fig. 1a) [13]. Cacosmia may be the result of purulent secretions associated with growth of Staphylococcus aureus or Pseudomonas aeruginosa. Epiphora may be seen as an early sign caused by involvement of the nasolacrimal duct and the lacrimal sac, due to direct granulomatous involvement, infection, or compression caused by nasal inflammation [14,15].

In active nasal disease, the nasal mucosa displays diffuse hemorrhage, crusting and purulent secretions. These conditions cause nasal obstruction. Nasal mucosal manifestations may be more or less severe, and patients may relate only nasal pain [13]. The most common site of active nasal disease is the anterior portion of the nasal septum, where vessels converge to supply the septal cartilage (Fig. 1b). Nasal septal perforation generally begins in this area and may progress to involve the entire cartilage. On occasion, edema of the mucosa and crusting are so severe that the perforation does not become apparent until the disease is brought into remission and the diseased tissue is absorbed [10]. The remaining mucosa and other structures of the nose, e.g., turbinates, are also frequently affected.

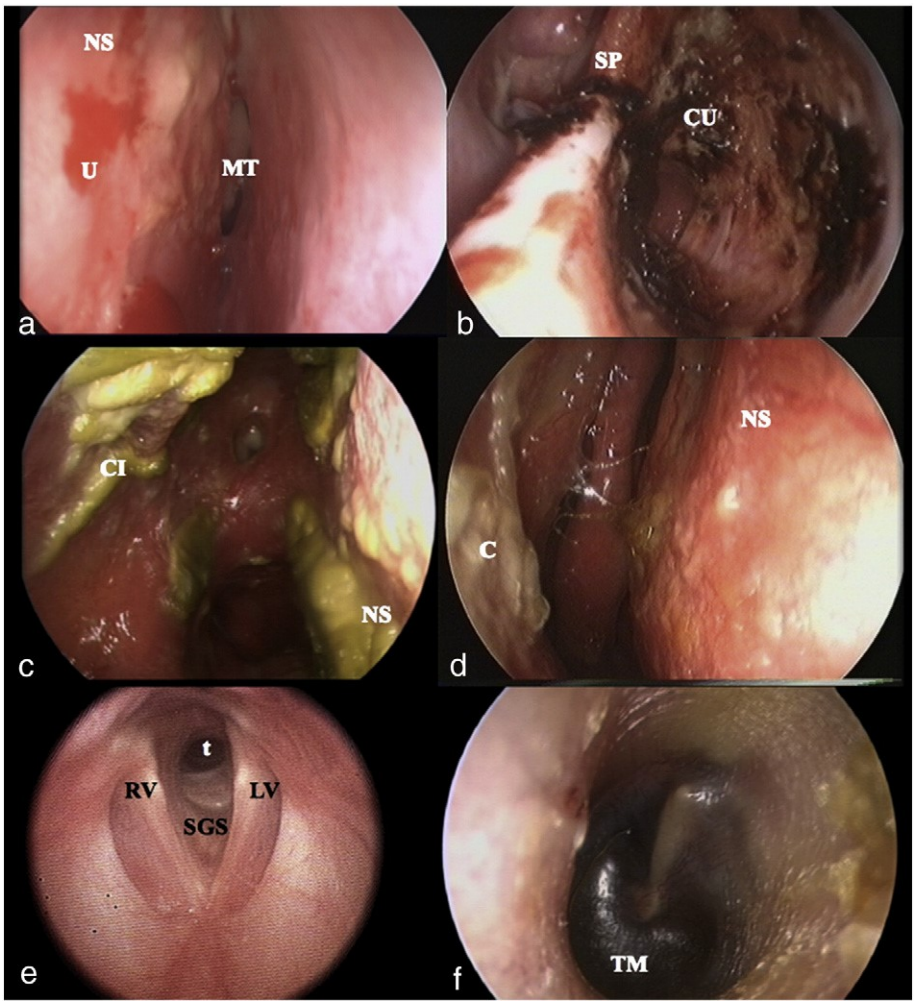


Fig 1. Endoscopic features in GPA patients: a) left nasal cavity with an ulcer (u) on the nasal septum (NS) and the middle turbinate (MT). b) Left nasal cavity with septal perforation and diffuse crusting with ulcers (CU). c) Right nasal cavity showing diffuse crusting with infection. d) Right nasal cavity with crusting in a patient with remission. e) Subglottic stenosis (SGS) with normal right (RV) and left (LV) vocal folds; narrowed tracheal opening (t). f) Serous otitis media, tympanic membrane (MT).

Less frequently, destruction of the nose may lead to a “typical” GPA saddle nose deformity [13].

Paranasal sinuses are frequently involved when examined by computed tomography (CT) or magnetic resonance imaging (MRI) [16,18]. The nasal mucosa is frequently preferred for diagnostic biopsies as it can be performed under local anesthesia, but the maxillary and ethmoid sinuses are also excellent targets (granulomatous inflammation) for representative biopsies. CT or MRI does not allow the distinction of granulomatous inflammation from non-specific inflammation or infection during the acute stage [17]. Thus, sinus involvement may be over-reported at centers using these scans routinely. In the chronic stages of disease, especially after several relapses, the sinuses become filled with scar tissue and the maxillary sinuses frequently become smaller, with progressive ossification of the maxillary bone. These changes can be easily detected by MRI [17].

In cases of extensive nasal mucosal involvement, endoscopic sinus surgery should be performed with caution in order to not add to the destructive potential of the disease, with an increase in nasal space and crust formation.

In the management of nasal damage, septal perforation, saddle nose deformity and epiphora become major challenges. Septal perforations are generally not treated [13,18]. Surgery of saddle nose deformity with implantation of cartilage or bone can be performed in patients who have achieved stable longstanding remission [19]. However, the loss of soft tissue, along with nasal support structures, often tends to reshape the saddle nose over time [20]. Dacryocystorhinostomy for epiphora and/or recurrent infection in the lacrimal sac can be performed using an external approach and/or endonasal procedures [14,15]. The major problem is infection and necrosis of the surgical repair area as noses with chronic crusts and/or superinfection pose specific problems.

Destruction of nasal ciliated epithelium changes mucus transport and favors chronic infection with crust formation (Fig. 1c,d) that can be managed with various forms of saline nasal irrigation combined with nasal ointment to soften crusts [13].

2.1. Ear

Otologic manifestations are common during the clinical course of generalized GPA, and their prevalence varies from 19% to 61% [21,22]. An otologic disorder may be part of the initial clinical presentation of GPA in about 33% of cases [23]. The external ear is rarely involved in GPA. Disease manifestations may include erythematous or ulcerated lesions resembling perichondritis. When involving the external canal, lesions may mimic otitis externa, but are generally not responsive to antibiotic or local steroid therapy [24,25].

The most common otological manifestation of GPA is unilateral or bilateral serous, less frequently purulent, otitis media (Fig. 1f). Eustachin tube dysfunction resulting from nasal inflammation and obstruction is a significant predisposing factor for otitis media [26].

More rarely, patients present with granulomatous involvement of the middle ear and mastoid cavity, lesions which occasionally can be observed through the tympanic membrane. Since a middle ear disorder may be the initial presentation of GPA in some patients, it is important to consider GPA as a possibility in atypical or prolonged subacute inflammatory states of the ear. Above all, GPA should be suspected in patients in whom discrepancies between otoscopic findings and severe mixed hypoacusis are noted [27,29].

Different authors emphasize that surgical intervention in these cases should be minimal and performed only for diagnostic purposes, particularly during the acute stage [28].

Another important manifestation of GPA is progressive, initially high pitched sensorineural hearing loss, often accompanied by tinnitus; it was reported in around one-third of subjects in a study performed by the European Vasculitis Study Group (EUVAS). More rarely, the vestibule may be involved, leading to an acute onset of vertigo [13].

The pathogenetic mechanisms of GPA are not yet clear. One hypothesis is that hearing loss is related to the toxic action of inflammatory products in the middle ear. In a recent publication, a case of a rapidly progressive hearing loss occurring during a chronic otitis media with effusion was reported; in this patient, T1-weighted MRI of the labyrinth revealed bilateral cochleitis, demonstrated by a gadolinium enhancement of the basal cochlear turn [30]. On the other hand, both central and peripheral nervous systems are often involved in GPA. Consequently, cranial nerve palsies can result from compression by granulomatous lesions or from vasculitis of the vasa nervorum; 8th nerve damage may occur following CNS vascular damage [31,32].

Finally, a small percentage of patients suffer from 7th nerve palsy, most frequently in association with middle ear disorder [32].

2.2. Oral cavity

Oral lesions as a presenting feature of GPA have been reported in around 2% of cases and appear in about 5–10% of GPA patients during clinical anamnesis. Nonetheless, the American Academy of Rheumatology considers them to be distinctive diagnostic criteria for GPA, and their recognition is of utmost importance for timely diagnosis [33]. Oral lesions include mucosal ulcers on the tongue, cheeks and palate [34–36] as well as “strawberry” gingival hyperplasia [33,34]. The latter consists of characteristic irritating exophytic gingival swelling of reddish purple color with petechial hemorrhages that resemble complex strawberries [37]. The differential diagnosis of mucosal ulcers should include Crohn's disease, sarcoidosis, mycobacterial infections and drug abuse [38].

2.3. Salivary glands

Involvement of the large salivary glands, presenting as swellings of the submandibular or the parotid glands, is another rare manifestation of GPA [39]. Symptoms may be vague or mimic malignancy. When disease of the major salivary glands is the first presentation of GPA the cause may remain unknown until more typical other organ features arise or the diagnosis is established histopathologically. In order to avoid delays in the diagnosis it is important to recognize that salivary gland involvement may be the initial presentation of the disease.

Recent publications have focused on the possibility that gland resection is often inconclusive, and histology may report only nonspecific inflammation; moreover, preoperative biopsies showing concomitant vasculitis, necrosis and granulomatous inflammation diagnostic of GPA is present in only 16% of cases, whereas vasculitis and granulomatosis is present in 21%, and isolated necrosis in 23% of biopsies [40,41].

2.4. Laryngeal and tracheal manifestations

Airway involvement is found in 15–55% of patients with GPA [42–45]. On rare occasions, however, airway involvement may be the only or the presenting feature of GPA. Laryngeal and tracheal ulcers may be present in 25% of patients, but subglottic stenosis (Fig. 1e) is considered to be less common and is present in 16% according to the experience of the National Institutes of Health; half of these required tracheostomy [46]. A patient who develops severe subglottic stenosis that goes untreated might develop severe airway obstruction, which can rapidly become fatal if not treated [47]. The etiology of subglottic stenosis in GPA is not clearly understood. This region is the junction between two embryological growth centers and presents two different microcirculation pathways [48]. The stenoses are generally localized at the junction of this region and the true vocal folds, while the trachea above and below the stenosis are generally intact [48].

Common symptoms include hoarseness, cough, hemoptysis, dyspnea, stridor and wheezing [42,43,49] . Hoarseness and cough are

less frequent then dyspnea and wheezing, and are reported in less than 10% of patients with airway stenosis caused by GPA [42].

Management changes according to the type of stenosis, although treatment of subglottic stenosis remains a therapeutic dilemma [46]. Almost all non-medical therapies represent an invasive procedure. There are different types of treatment including translesional injection of corticosteroids, balloon dilatation, laser ablation, intraluminal stent placement, surgical resection and reanastomosis [49–54]. Short stenosis due to acute disease can be treated with standard medical therapy with the addition of inhaled topical glucocorticoids; laser therapy may be appropriate in some patients [13]. The complete removal of the stenosis leads to further stenosis, and thus laser therapy is used with radial incisions to reduce the tension on the surface of the stenosis. Dilatation therapy is effective only in rare cases of a thin web [53].

When chronic damage results in fixed lesions, surgical repair may be necessary to avoid chronic tracheostomy [46]. Different grafting techniques are available when the stenoses are localized to the cricoid [13,53]. Stenting may sometimes be necessary during the healing phase (3–6 months), or alternatively the tracheostomy can be kept to allow secure air passage through the larynx [55].

Three overriding messages about tracheobronchial lesions should be remembered. First, any surgical intervention should ideally be delayed until after the acute inflammation has resolved under medical therapy. Second, additional tissue injury resulting from the procedure should be minimized as much as possible. Third, if stents are used in selective patients, only silastic airway stents should be used and metal wire mesh stents should never be used in patients with GPA.

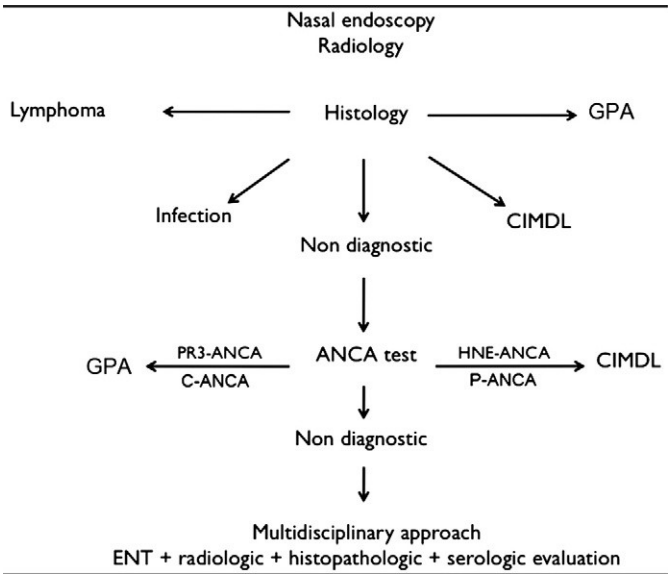
3. Differential diagnosis

GPA should enter in differential diagnosis with ulcerative lesions of the head and neck (Table 1). Infectious, inflammatory, neoplastic and substance abuse conditions can result in granulomatous inflammation and can also be destructive. Patients need to be evaluated with a careful history and physical examination to look for evidence of disease that is not localized to the head and neck region. Examination features common to this condition include refractory inflammation with nasal crusting, bleeding and friable mucosa, often with necrosis of the nasal septum and occasionally with extension into adjacent structures. All patients presenting with features suggesting the possibility of GPA should undergo flexible endoscopy and imaging with CT and /or MR to assess disease activity and extent and to identify the best region to biopsy. At the same time, serologic examination is very important, and in some cases can by itself point towards the correct diagnosis, highlighting the importance of a multidisciplinary approach to GPA (Table 2).

Table 1
Differential diagnosis in head and neck granulomatous lesion.

Infection	Inflammation	Neoplasm
Bacterial	Wegener granulomatosis	NK-T cell Lymphoma
Tuberculosis	Sarcoidosis	(midline lethal granuloma)
Leprosy	Churg–Strauss syndrome	
Syphilis	Eosinophilic granulomatosis	
Rhinoscleroma	CIMDL	
Actinomicosis		
Fungal		
Aspergillosis		
Zygomycosis		
Dermatacietes		
Rhinosporidiosis		
Blastomycosis		
Histoplasmosis		
Sporotrichosis		
Coccidioidomycosis		
Protozoal		
Leishmaniasis		

Table 2
Diagnostic work-up.



4. Concluding remarks

The otorhinolaringologist is often one of the first physicians to patients with GPA. The recognition of signs and symptoms of GPA affecting the upper respiratory tract is crucial for an effective diagnostic evaluation and to readily arrive at the proper diagnosis that allows the timely initiation of appropriate therapy. The frequent follow-up of these patients is necessary to diagnose early relapses and to minimize damage at any of the affected sites.

Take-home message

- There is no single diagnostic test for the limited forms of GPA. Rather diagnosis is based on a combination of clinical, endoscopic, radiologic, histologic and serologic evaluations.
- A limited form of GPA must always be suspected in recurrent infections of the nose, paranasal sinuses, otitis, facial palsy, mucosal ulcers and laryngitis.
- The otorhinolaryngologist is often the first clinician to diagnose a limited form of GPA.

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Corrigendum

granulomatosis with polyangiitis (Wegener's)" Corrigendum to "Otorhinolaryngological manifestations in [Autoimmun. Rev. 12 (2013) 501–505]

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Functional Characterization of Antineutrophil Cytoplasmic Antibodies in Patients With Cocaine-Induced Midline Destructive Lesions

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Objective. **Antineutrophil cytoplasmic antibodies (ANCA) binding to neutrophil elastase (NE) and proteinase 3 (PR3) are detectable in most patients with cocaine-induced midline destructive lesions (CIMDL), but the pathogenic role and antigen specificity of these antibodies are unknown. This study was undertaken to assess the effects of NE ANCA on the enzymatic activity of NE, to determine whether these antibodies interfere with the physiologic effect of secretory leukoprotease inhibitor (SLPI), and to investigate the antigen specificity of both NE and PR3 ANCA in patients with CIMDL. We also compared the binding of PR3 ANCA in patients with CIMDL with that in patients with Wegener's granulomatosis (WG).**

Methods. **PR3 ANCA and NE ANCA were detected by capture enzyme-linked immunosorbent assays (ELISAs) and by indirect immunofluorescence. IgG was purified from the patients' sera, and the influence of NE ANCA on the enzymatic activity of NE and on the inhibitory activity of SLPI was investigated by determining the hydrolysis of *N*-methoxysuccinyl-Ala-AlaPro-Val *p*-nitroanilide by NE.**

Results. **IgG from NE ANCA–positive sera of patients with CIMDL inhibited the enzymatic activity of NE and did not interfere with the activity of SLPI. In contrast to the findings in WG sera, measurement of PR3 ANCA in CIMDL sera showed only fair to moderate concordance between the 2 different capture ELISAs. Cross-inhibition experiments demonstrated that NE ANCA and PR3 ANCA represent distinct autoantibodies in patients with CIMDL.**

Conclusion. **The functional effects of NE ANCA on the enzymatic activity of NE or on the activity of SLPI cannot be implicated in the pathogenesis of CIMDL. The autoimmune reaction that targets neutrophil serine proteases in patients with CIMDL is frequently directed against more than one antigen. The ANCA response, including the reactivity of PR3 ANCA, in patients with CIMDL differs from what has been described in patients with WG.**

Cocaine-induced midline destructive lesions (CIMDL) are an uncommon complication of habitual intranasal cocaine insufflation (1). Patients with CIMDL develop extensive destruction of the midfacial osteocartilagenous structures, which resembles involvement of the upper respiratory tract in patients with Wegener's granulomatosis (WG) (1).

Antineutrophil cytoplasmic antibodies (ANCA) are detectable in most patients with WG as well as in most patients with CIMDL. Whereas a cytoplasmic (cANCA) staining pattern predominates in WG, a perinuclear (pANCA) staining pattern is typical in CIMDL. In addition, most sera from patients with CIMDL react with human neutrophil elastase (NE) (1). ANCA specific for proteinase 3 (PR3), the typical finding in the sera of patients with WG, are present in 50% of patients with CIMDL (1). ANCA specific for NE repre-

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sent a valuable diagnostic marker for CIMDL and are almost never detectable in patients with WG or in those with microscopic polyangiitis (MPA) (1).

It remains unknown whether ANCA contribute to the pathogenesis of CIMDL. Moreover, it has yet to be determined if the simultaneous recognition of both types, NE ANCA and PR3 ANCA, is attributable to cross-reaction of the antibodies or to the coexistence of distinct antigen-specific antibodies. An enzyme activity–enhancing effect of NE ANCA isolated from patients with MPA and from patients with osteomyelitis has been reported (2). Therefore, we investigated whether the binding of these ANCA to NE enhances the proteolytic activity of NE and/or interferes with its major physiologic antagonist, secretory leukoprotease inhibitor (SLPI) (3), on the mucosal surfaces of the upper respiratory tract. We also explored whether, in the sera of patients with CIMDL, the reacting NE ANCA and PR3 ANCA are cross-reacting antibodies, or whether each represents a distinct antibody population.

PATIENTS AND METHODS

Sample collection. Serum samples from 37 patients with CIMDL evaluated at Brescia University in Italy and at the Mayo Clinic in Rochester, Minnesota were analyzed. Samples from 5 healthy volunteers and from 5 cANCA-positive and PR3 ANCA–positive patients with WG served as controls. The Mayo Clinic Institutional Review Board approved the study. Due to the limited quantities of serum available, not all samples were included in all experiments.

ANCA testing. All serum samples from the patients with CIMDL were evaluated with multimodality ANCA testing. ANCA staining patterns were assessed by indirect immunofluorescence (IIF) on ethanol-fixed neutrophils at serum dilutions of 1:4 and 1:16 (1). PR3 ANCA were measured by commercial direct enzyme-linked immunosorbent assay (ELISA), as well as by a capture ELISA with the MCPR3-2 capturing antibody and a PR3 anti–*c-myc* capture ELISA (4,5). NE ANCA were determined using a sheep anti-NE capture ELISA (using polyclonal sheep anti-NE capturing antibody) and an NE anti–*c-myc* capture ELISA (1,5). In the anti–*c-myc* assays, recombinant *c-myc*–tagged antigens were captured by an anti–c-*myc* antibody (P2241; Sigma-Aldrich, St. Louis, MO) (5).

IgG preparation. IgG was purified by applying 0.5 ml of serum to a protein G column (Pierce, Rockford, IL), according to the manufacturer's instructions. The fractions with the highest spectrophotometrically measured absorbance (280 nm; A_{280}) were pooled. The optical density (OD) of the pooled sample was measured at 280 nm, and the IgG concentration was calculated as follows: IgG (in mg/ml) (A_{280}/ 14) 10.

Effect of NE ANCA–containing IgG on the hydrolytic activity of NE. To determine the effect of NE ANCA on the hydrolysis of the peptide substrate *N*-methoxysuccinyl-AlaAla-Pro-Val *p*-nitroanilide (MeAAPV; Sigma-Aldrich), 12.5 ng of NE per well (Athens Research Technology, Athens, GA) was diluted in 25 l of buffer (1% Triton X-100, 0.1*M* Tris, pH 8.0), followed by the addition of 25 g of IgG in each well. Samples (50 l/well) were incubated for 30 minutes at 37°C, and 50 l of 2 m*M* MeAAPV in DMSO was added. The change in the OD per minute (mOD/minute) was measured every 30 seconds for 10 minutes, at 405–490 nm. Results are expressed as a percent of the values for the positive control (12.5 ng NE/well, MeAAPV in the absence of IgG).

Effect of NE ANCA–containing IgG on the inhibition of the hydrolytic activity of NE by SLPI. To determine whether NE ANCA interfere with the inhibition of NE-mediated hydrolysis of MeAAPV by SLPI, 12.5 ng of NE/well was diluted in 20 l of buffer (1% Triton X-100, 0.1*M* Tris, pH 8.0). Twenty-five micrograms of IgG was diluted in 20 l of the same buffer and added to the NE in each well. Samples were incubated for 30 minutes at 37°C; 10 l of SLPI (R&D
Systems, Minneapolis, MN), diluted 1:100 in buffer (1% Triton X-100, 0.1*M* Tris, pH 8.0), and 50 l of 2 m*M* MeAAPV in DMSO were added; this concentration of SLPI was previously determined to be sufficient to completely inhibit the enzymatic activity of 12.5 ng of NE. The mOD/minute was then measured every 30 seconds for 10 minutes, at 405–490 nm. Results were normalized by subtracting the values for the positive control (12.5 ng of NE and 10 l of SLPI). This was followed by calculation of the residual enzymatic activity of NE, expressed as a percent of the values for the negative control (12.5 ng of NE without SLPI).

ANCA cross-reactivity. Serial dilutions of all serum samples that were found positive for the antibodies by the PR3 anti–*c-myc* capture ELISA and the NE anti–*c-myc* capture ELISA were analyzed. For each sample, the dilution yielding 50% of the maximum OD (OD_{50}) was determined for each ANCA type. All subsequent experiments were conducted using this dilution. All assays were performed in duplicate.

Anti–*c-myc*–coated plates were loaded with a saturating dilution of serum-free medium supernatant of 293 cell clones expressing enzymatically inactive recombinant PR3 *c-myc* or NE *c-myc* for 1 hour at room temperature. Antigenfree control wells (anti–*c-myc*–coated plates incubated with serum-free medium supernatant from sham-transfected 293 cells, diluted 1:2 in IRMA buffer) served as negative controls. After 3 washes with 20 m*M* Tris, 500 m*M* NaCl, pH 7.5, and 0.05% Tween, 100 l of patient sera per well, diluted in Tris buffered saline Immulite and 0.5% bovine serum albumin to the predetermined 50% saturation point, was preadsorbed for 24 hours at 4°C in antigen-coated wells and control wells. Following this preadsorption, the supernatants were analyzed by PR3 anti–*c-myc* capture ELISA and NE anti-*c-myc* capture
ELISA (5).

Statistical analysis. GraphPad Prism 4.0 for Macintosh (GraphPad Software, San Diego, CA) was used for the statistical analyses. Differences in the ODs and changes in the enzymatic activity were compared with

Wilcoxon's signed rank test and the Mann-Whitney U test, respectively. *P* values less than 0.05 were considered significant. Concordance was assessed using kappa statistics.

RESULTS

Effects of NE ANCA–containing IgG from patients with CIMDL on the enzymatic activity of NE. To determine whether NE ANCA from patients with CIMDL enhance the enzymatic activity of NE, we measured the effect of NE ANCA–containing IgG on the hydrolysis of MeAAPV by NE. Of the 37 patients with CIMDL, 4 were excluded because their sera tested negative for the ANCA, 2 were excluded because of the detection of exclusively PR3 ANCA, and 5 were excluded because the quantity of serum needed to extract IgG was insufficient. Consequently, 26 serum samples were included in this experiment. Multimodality ANCA testing revealed that 16 of these serum samples were positive for both NE ANCA and PR3 ANCA, and 10 were positive for NE ANCA only. None of these IgG preparations enhanced the hydrolytic activity of NE.

Of the 26 CIMDL serum samples, IgG preparations from 16 samples inhibited the NE enzymatic activity by more than 20%. There was no difference in the extent of inhibition between the samples positive for both NE ANCA and PR3 ANCA and those positive for NE ANCA only. NE activity was decreased by more than 20% in only 1 of the 5 normal control IgG preparations and in none of the 5 PR3 ANCA–positive IgG preparations of samples from patients with WG (Figure 1A). These data indicate that NE ANCA–containing IgG from patients with CIMDL does not enhance, but rather inhibits the hydrolytic activity of NE.

Effect of NE ANCA–containing IgG on the inhibitory activity of SLPI in patients with CIMDL. NE ANCA could interfere with the physiologic inhibition of NE activity on mucosal surfaces in patients with CIMDL. We therefore investigated the influence of NE ANCA–containing IgG on the inhibition of the enzymatic activity of NE by SLPI. Only 23 of the 26 CIMDL serum samples used in the previous experiment were available in sufficient quantity to be included in this experiment. On multimodality ANCA testing, 15 samples were positive for both NE ANCA and PR3 ANCA, and 8 were positive for NE ANCA only.

Our results showed that there was no interference with the inhibitory activity of SLPI on the hydrolysis of MeAAPV by NE in any of the 23 CIMDL serum samples, similar to the results obtained in the 5 WG serum samples and in the 5 normal control samples.

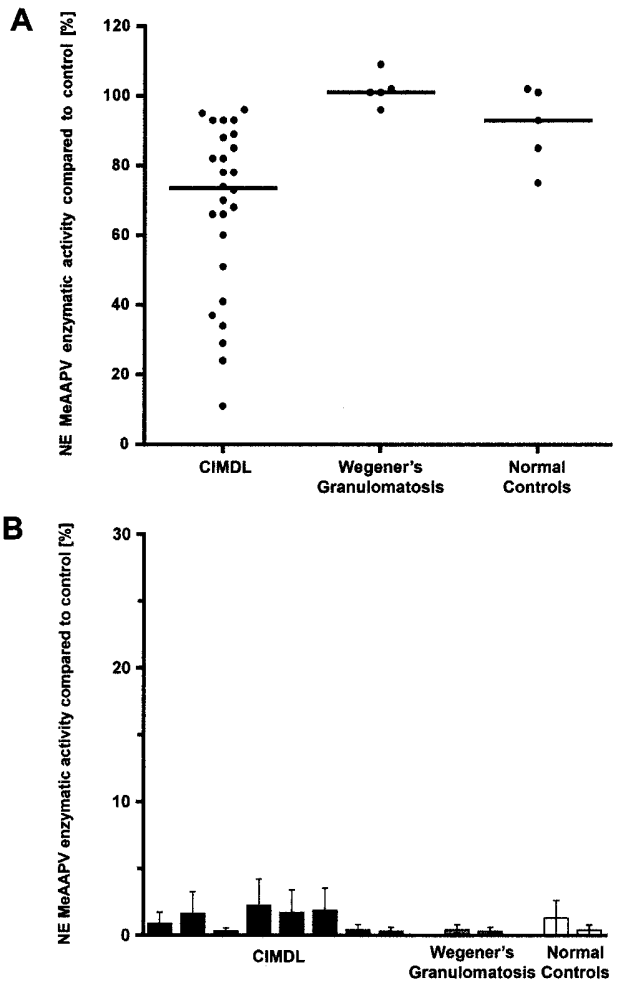

Figure 1. A, Proteolytic digestion of *N*-methoxysuccinyl-Ala-Ala-ProVal *p*-nitroanilide (MeAAPV) by neutrophil elastase (NE) in the presence of IgG prepared from 26 serum samples positive for NE antineutrophil cytoplasmic antibodies (ANCA) from patients with cocaine-induced midline destructive lesions (CIMDL), 5 proteinase 3 (PR3) ANCA–positive serum samples from patients with Wegener's granulomatosis (WG), and 5 normal control serum samples. The enzymatic activity of NE was significantly lower when NE was incubated with IgG prepared from the CIMDL sera as compared with IgG prepared from the PR3 ANCA–positive WG sera (*P* 0.001) and normal control sera (*P* 0.03). Horizontal lines represent the median. **B,** Residual enzymatic activity of 12.5 ng of NE (a sufficient concentration for complete inhibition) incubated with secretory leukoprotease inhibitor in IgG samples from 8 representative patients with CIMDL, 2 patients with WG, and 2 normal controls. Bars show the mean and SEM results from 3 experiments.

Furthermore, no difference was noted between the CIMDL serum samples positive for both NE ANCA and

PR3 ANCA and the samples positive for NE ANCA only. Representative results are shown in Figure 1B.

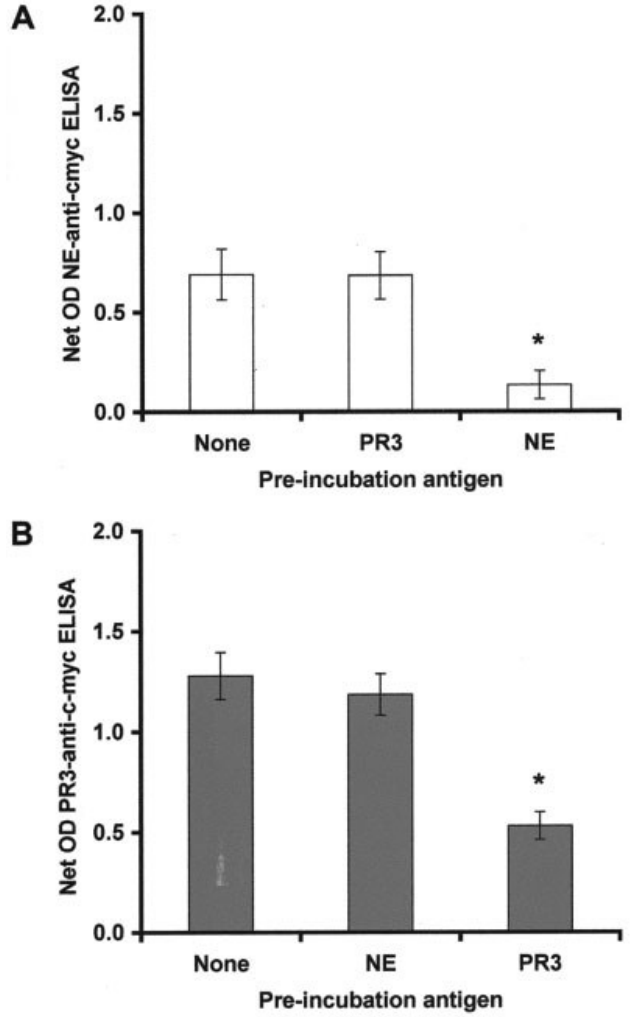

Figure 2. Cross-reactivity experiments with NE ANCA and PR3 ANCA in a representative serum sample from a patient with cocaineinduced midline destructive lesions. Following preincubation with NE, PR3, or no antigen, the serum sample was analyzed with the NE anti–*c-myc* enzyme-linked immunosorbent assay (ELISA) **(A)** and the PR3 anti–*c-myc* ELISA **(B)**. There was no statistically significant change in optical density (OD) when PR3 ANCA reactivity was measured following preadsorption with NE, and vice versa. In contrast, there was a significant decrease in net OD for NE ANCA **(A)** and PR3 ANCA **(B)** after preincubation with PR3 and NE, respectively. *P* 0.03 versus the other antigen groups. Bars show the mean SEM from 3 experiments. See Figure 1 for other definitions.

Distinguishing PR3 ANCA in patients with CIMDL from PR3 ANCA in patients with WG. We also explored whether the PR3 ANCA in the sera of patients with CIMDL could be distinguished from the PR3 ANCA in the sera of patients with WG. Most capture ELISAs for detection of PR3 ANCA use monoclonal antibodies to capture the antigen, such as the monoclonal antibody against PR3 used in our MCPR3-2 capture ELISA (4); alternatively, *c-myc*–tagged recombinant antigens can be captured by antibodies recognizing the *c-myc* tag (5). We previously demonstrated very good concordance (99%; 0.98) between the results obtained with each type of assay in samples from patients with WG (6). In contrast, in the 37 serum samples from patients with CIMDL, only a fair to moderate concordance (70% [26 of 37], 95% confidence interval 54– 83%; 0.42) between the 2 assays for PR3 ANCA was found. These observations suggest that the PR3 ANCA in patients with CIMDL target different epitopes than those targeted by the PR3 ANCA in patients with WG.

IIF was used to determine the ANCA staining pattern in the 26 CIMDL serum samples included in the function studies. In contrast to the typical cANCA pattern seen in WG sera, a pattern of pANCA staining

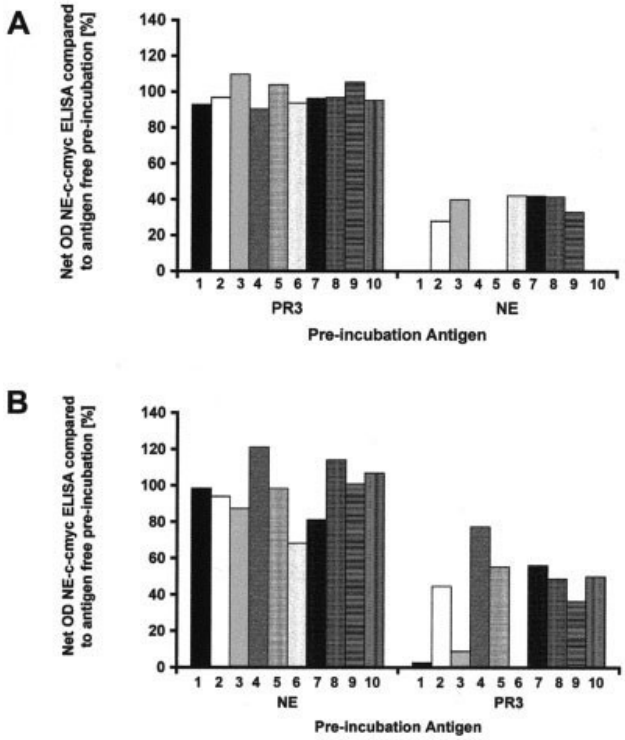

Figure 3. Cross-reactivity experiments in all 10 double-positive CIMDL serum samples. Results are the net optical density (OD) following preincubation with either NE or PR3, tested with the NE anti–*c-myc* enzyme-linked immunosorbent assay (ELISA) **(A)** and the PR3 anti–*c-myc* ELISA **(B),** compared with the values from controls preincubated without antigen. The interassay variability of the anti–*cmyc* capture ELISAs used in this experiment varies between 15.8% and 18.4% (see ref. 6). See Figure 1 for other definitions.

was observed in the majority of the CIMDL sera (92.3% [24 of 26]), including all 10 samples that were positive for

NE ANCA and 14 samples that reacted with both NE and PR3 by antigen-specific capture ELISAs. Only 2 patients double-positive for NE ANCA/PR3 ANCA had a cANCA IIF staining pattern. When tested by direct ELISA for PR3 ANCA, 11 (42%) of the 26 CIMDL serum samples tested positive for PR3 ANCA.

Cross-reactivity of NE ANCA and PR3 ANCA in patients with CIMDL. Of the 37 CIMDL serum samples, 21 were NE ANCA positive by the NE anti–*c-myc* capture ELISA. Of these, 10 were also PR3 ANCA positive by the PR3 anti–*c-myc* capture ELISA. All 10 of these NE ANCA/PR3 ANCA double-positive samples were included in the cross-inhibition experiments.

To identify cross-reactivity between NE ANCA and PR3 ANCA, the CIMDL serum samples were incubated for 24 hours with PR3 or NE, or left without antigen. Following this preadsorption, the samples were reanalyzed by NE anti–*c-myc* capture ELISA and PR3 anti–*c-myc* capture ELISA. As expected, a significant reduction in ANCA reactivity was detected for NE ANCA following preadsorption with NE and for PR3 ANCA following exposure to PR3 (Figure 2). In contrast, the reactivity of NE ANCA was not significantly affected by preadsorption with PR3, or vice versa. Similar results were obtained for all 10 double-positive CIMDL samples included in this analysis (Figure 3). These data demonstrate that double-positive sera (for both NE ANCA and PR3 ANCA) from patients with
CIMDL contain at least 2 distinct antibody specificities, rather than a single antibody cross-reacting with both NE and PR3.

DISCUSSION

NE ANCA from patients with MPA and from those with chronic staphylococcal osteomyelitis have been reported to enhance the enzymatic activity of NE (2). We speculated that this mechanism might also contribute to the pathogenesis of CIMDL. However, our data demonstrated an inhibitory effect of NE ANCA on NE enzymatic activity and revealed that there was no interference by NE ANCA with the activity of SLPI. These differences could be explained by the binding of different epitopes by NE ANCA in patients with CIMDL as compared with that observed in other diseases. Furthermore, our investigations examined the effect of the entire NE ANCA–containing IgG fraction, whereas the previous study examined the influence of affinity-purified NE ANCA (2). Given that, in vivo, patient sera contain a mixture of various antibodies rather than antibodies that target only a specific antigen, we believe that our approach more realistically reflects the interactions among the various antibodies. The cleavage of large molecular substrates of NE, such as elastin, is more sensitive to the effects of inhibitors than is the hydrolysis of MeAPPV by NE. Consequently, experiments with large molecular substrates were not pursued once we identified an inhibitory, rather than enhancing, effect for most of these NE ANCA.

Although our study shows that NE ANCA in patients with CIMDL do not enhance the enzymatic activity of NE, we cannot exclude the possibility that they are still involved in the pathogenesis of CIMDL through different mechanisms. Similar to the observed effects of PR3 ANCA and ANCA against myeloperoxidase (MPO) in patients with ANCA-associated vasculitides, NE ANCA may activate primed neutrophils, modify the clearance of apoptotic cells, or enhance and/or perturb neutrophil apoptosis (7,8). Interestingly, a high frequency of apoptotic epithelial cells has been described in CIMDL (6).

Autoantibodies binding to both NE and PR3 are frequently detected in patients with CIMDL (1). Our cross-inhibition experiments clearly showed that the sera from patients with CIMDL that were double-positive for NE ANCA and PR3 ANCA contained at least 2 antigenspecific antibodies recognizing unique epitopes, rather than shared epitopes of NE and PR3.

The pANCA pattern is the predominant IIF staining pattern in patients with CIMDL, even in most of the CIMDL serum samples that also test positive for PR3 ANCA by direct or capture ELISAs (1). Concurrently, MPO ANCA are usually absent in CIMDL sera, suggesting that NE ANCA are responsible for most of the pANCA immunofluorescence staining.

Moreover, we demonstrated that detection of PR3 ANCA by antigen-specific capture ELISAs varies between sera from patients with CIMDL and sera from patients with WG (5). This is likely caused by competition of PR3 ANCA with the capturing antibody for specific target epitopes in selected patients with CIMDL, but not in patients with WG. Furthermore, whereas IgG preparations from many PR3 ANCA– positive patients with WG either inhibit or enhance the enzymatic activity of PR3 in the hydrolysis of MeAAPV, no change in the hydrolytic activity of PR3 was detected in experiments with IgG preparations from NE ANCA– and PR3 ANCA–positive CIMDL sera (Specks U, et al: unpublished observations). These results suggest that the nature of the antigen, and possibly the specific epitopes recognized by the specific autoantibodies, may determine the disease phenotype.

In conclusion, our data indicate that the functional effects of NE ANCA on the activities of NE or SLPI are unlikely to contribute to the pathogenesis of CIMDL. Moreover, despite some clinical similarities with the nasal lesions of WG, the autoantibody characteristics encountered in CIMDL are distinct from those of WG.

AUTHOR CONTRIBUTIONS

Dr. Specks had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Study design.** Peikert, Specks.
Acquisition of data. Peikert, Hummel, McKenney, Gregorini, Trimarchi, Specks.
Analysis and interpretation of data. Peikert, Finkielman, Hummel, McKenney, Gregorini, Trimarchi, Specks.
Manuscript preparation. Peikert, Finkielman, Specks.
Statistical analysis. Peikert, Finkielman, Specks.

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