Progress in Neuroscience

An interdisciplinary journal

For specialists in:

NEUROLOGY

NEUROSURGERY

NEURORADIOLOGY

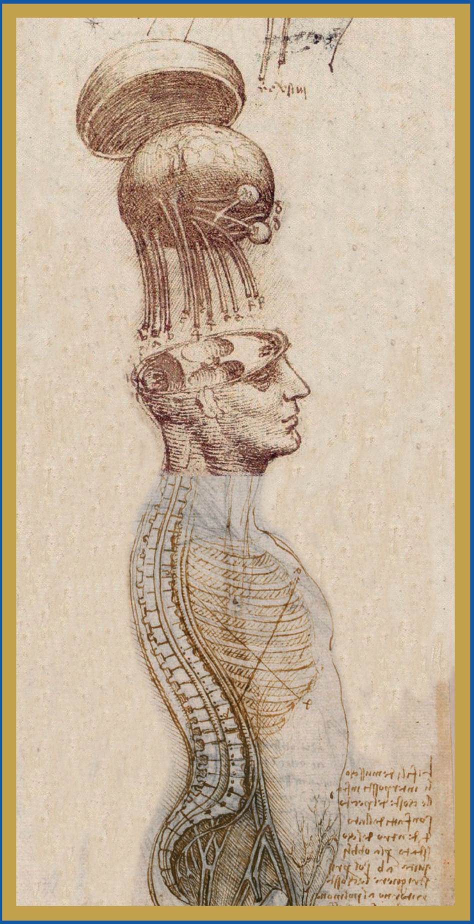
NEUROONCOLOGY

NEUROPATHOLOGY

NEUROANAESTHESIA

NEUROREHABILITATION

and related disciplines



Volume 6
Issue 1-4
2021

SNO
Official Journal

ISSN 2240-5127 (print)
www.progressneuroscience.com
Authorized by the Tribunale in Trento, 11 maggio 2011 - Registration: Registro della Stampa n. 18 and Registro degli Operatori di Comunicazione (ROC) n. 3810 - *Quarterly* -
€ 20,00

Progress in
Neuroscience

An interdisciplinary journal

SNO Scienze Neurologiche Ospedaliere
Official Journal

ISSN 2240-5127
Volume 6, Issue 1-4, March-December 2021

INDEX

Editorial

Cognitive lock-down

Correspondence: Dr. Bruno Zanotti, Neurosurgery Unit, Neuroscience Department, “C. Poma” Hospital, 46100 Mantua, Italy, bruno.zanotti@rivistamedica.com
Progress in Neurosciences 2021; 6 (1-4): 3-17. *ISSN: 2240-5127.*

REFERENCES

1. SNO. Statuto dell'Associazione "Società dei Neurologi, Neurochirurghi e Neuroradiologi Ospedalieri" [cited 2022, May 20]. Available from: snoitalia.org/la-societa/lo-statuto/
2. SNO News. "Io mi vaccino" - La SNO promuove e incoraggia la vaccinazione consapevole e responsabile per proteggere noi stessi e gli altri [cited 2022, May 20]. Available from: snoitalia.org/io-mi-vaccino-la-sno-promuove-e-incoraggia-la-vaccinazione-consapevole-e-responsabile-per-proteggere-noi-stessi-e-gli-altri/
3. Italian Government and Presidency of the Council of Ministers. PM Draghi's press conference with Justice Minister Cartabia and Health Minister Speranza - Introduction by PM Draghi. Wednesday, 28 July 2021 [cited 2022, May 20]. Available from: www.governo.it/it/articolo/conferenza-stampa-draghi-cartabia-speranza/17515
4. Istituto Superiore di Sanità. Il case fatality rate dell'infezione SARS-CoV-2 a livello regionale e attraverso le differenti fasi dell'epidemia in Italia (versione del 20 gennaio 2021). Rapporto ISS COVID-19, n. 1/2021 [cited 2022, May 20]. Available from: www.iss.it/documents/20126/0/Rapporto+ISS+COVID-19+n.+1_2021.pdf
5. Istituto Superiore di Sanità e ISTAT. Impatto dell'epidemia Covid-19 sulla mortalità totale della popolazione residente. Anno 2020 e gennaio-aprile 2021 (Report del 10 giugno 2021) [cited 2022, May 20]. Available from: www.istat.it/it/files//2021/06/Report_ISS_Istat_2021_10_giugno.pdf
6. Istituto Superiore di Sanità. Epicentro: L'epidemiologia per la sanità pubblica [cited 2022, May 20]. Available from: www.epicentro.iss.it/
7. Fondazione Centro Cardiologia e Cardiochirurgia A. De Gasperis. Le cause di morte in Italia [cited 2022, May 20]. Available from: https://www.degasperis.it/le-cause-di-morte-in-italia.html
8. Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro. Relazione Annuale 2021 del Presidente Franco Bettoni. Roma, 25 luglio 2022 [cited 2022, May 20]. Available from: https://www.inail.it/cs/internet/docs/alg-relazione-annuale-anno-2021.pdf
9. Istat e ACI. Incidenti stradali. Anno 2020 (22 luglio 2021) [cited 2022, May 20]. Available from: https://www.istat.it/it/files//2021/07/Incidenti-stradali_2020.pdf
10. Sangalli D, Martinelli-Boneschi F, Versino M, Colombo I, Ciccone A, Beretta S, Marcheselli S, Altavilla R, Roncoroni M, Beretta S, Lorusso L, Cavallini A, Prelle A, Guidetti D, La Gioia S, Santalucia P, Zanferrari C, Grampa G, D'Adda E, Peverelli L, Colombo A, Salmaggi A; SNO-COVID-19 group. Impact of SARS-CoV-2 infection on acute intracerebral haemorrhage in northern Italy. J Neurol Sci 2021; 426: 117479. doi: 10.1016/j.jns.2021.117479.
11. Colombo A, Martinelli Boneschi F, Beretta S, Bresolin N, Versino M, Lorusso L, Spagnoli D, Nastasi G, Vallauri D, Rota S, Repaci M, Ferrarini M, Pozzato M, Princiotta Cariddi L, Tabaee Damavandi P, Carimati F, Banfi P, Clemenzi A, Marelli M, Giorgianni A, Vinacci G, Mauri M, Melzi P, Di Stefano M, Tetto A, Canesi M, Salmaggi A. Posterior reversible encephalopathy syndrome and COVID-19: a series of 6 cases from Lombardy, Italy. eNeurologicalSci 2020; 22:100306. doi: 10.1016/j.ensci.2020.100306.
12. Ciccone A. SARS-CoV-2 vaccine-induced cerebral venous thrombosis. Eur J Intern Med 2021; 89: 19-21. doi: 10.1016/j.ejim.2021.05.026.
13. Ciccone A, Zanotti B; Working group on cerebral venous thrombosis after COVID-19 vaccination. The importance of recognizing cerebral venous thrombosis following anti-COVID-19 vaccination. Eur J Intern Med 2021: 89:115-117. doi: 10.1016/j.ejim.2021.05.006.
14. Pardi FP. Il vaso di pandora del coronavirus. MicroMega 2020; 4: 105-114.
15. Villa ML. La scienza sa di non sapere per questo funziona. goWare e Edizioni Angelo Guerini e Associati, Milano, 2016.
16. Beck U, Kropp C. Environmental risks and public perception (chapter 41). In J. Pretty, A.S. Ball, T. Benton, J.S. Guivant, D.R. Lee, D. Orr, M.J. Pfeffer, H. Ward (editors): The Sage handbook of environment and society. Sage Publication, Los Angeles, 2007.
17. Croce M., Salvatore A. Cos'è lo stato di eccezione. Nottetempo, Milano, 2021.
18. Weingart P. Paradox of scientific advising. In G. Bechmann, I Hronsk (editors): Expertise and its interfaces. The tense relationship of science and politics. Sigma, Berlin, 2003: 53-90.
19. Stilgoe J, Irwin A, Jones K. The received wisdom: opening up expert advice. Demos, London, 2006.
20. Cenzato M, DiMeco F, Fontanella M, Locatelli D, Servadei F. Neurosurgery in the storm of COVID-19: suggestions from the Lombardy region, Italy (ex malo bonum). J Neurosurg 2020 (Apr 10):1-2. doi: 10.3171/2020.3.JNS20960.
21. SIAARTI. Raccomandazioni di etica clinica per l'ammissione a trattamenti intensivi e per la loro sospensione, in condizioni eccezionali di squilibrio tra necessità e risorse disponibili. Version 01. Published March 6, 2020 [cited 2022, May 20]. Available from: www.flipsnack.com/siaarti/siaarti_-_covid19_-_raccomandazioni_di_etica_clinica_-2/full-view.html.
22. Verlicchi A, Zanotti B (editors), Soldano F, Bricolo A (collaborators): Lo stato vegetativo persistente: cosa ne pensano i medici italiani. Rivista Medica 2002, 8 (3-4): 53-130.
23. Verlicchi A, Soldano F, Zanotti B: Il pensiero medico nella gestione dello stato vegetativo. In: A. Verlicchi, B. Zanotti, M. Skrap (editors). Www.statovegetativo.it I

limiti della medicina che salva. New Magazine, Trento, 2006: 425-503.

24. Banchi a rotelle? Ora vanno in beneficenza nelle scuole del Congo [cited 2022, May 20]. Available from: www.leggo.it/italia/cronache/banchi_rotelle_da_padova_beneficenza_scuole_congo-6290855.html
25. Longo AR. Amori in emergenza. Mind Mente & Cervello 2021; 197: 24-31.
26. Pascolo PB, Berzuini C. Linee guida inerenti il problema della diluizione del COVID-19 nell'aria e il controllo del contagio. Dalla pratica al modello predittivo. Progress in Neuroscience 2020; 1-4 (Suppl. 1): 9-21.
27. Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in health care settings. Clin Infect Dis 2003; 37 (8): 1094-1101.
28. Tellier R. Review of aerosol transmission of influenza A virus. Emerg Infect Dis 2006; 12 (11): 1657-1662. doi: 10.3201/eid1211.060426.
29. Seto WH, Tsang D, Yung RW, Ching TY, Ng TK, Ho M, Ho LM, Peiris JS; Advisors of Expert SARS group of Hospital Authority. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). Lancet 2003; 361 (9368): 1519-1520. doi: 10.1016/s0140-6736(03)13168-6.
30. Orr NW. Is a mask necessary in the operating theatre? Ann R Coll Surg Engl 1981; 63 (6): 390-392.
31. Mitchell NJ, Hunt S. Surgical face masks in modern operating rooms - a costly and unnecessary ritual? J Hosp Infect 1991; 18 (3): 239-242. doi: 10.1016/0195-6701(91)90148-2.
32. Tunevall TG. Postoperative wound infections and surgical face masks: a controlled study. World J Surg 1991; 15 (3): 383-387. doi: 10.1007/BF01658736.
33. Oberg T, Brosseau LM. Surgical mask filter and fit performance. Am J Infect Control 2008; 36 (4): 276-282. doi: 10.1016/j.ajic.2007.07.008.
34. Fouladi Dehaghi B, Ghodrati-Torbati A, Teimori G, Ibrahimi Ghavamabadi L, Jamshidnezhad A. Face masks vs. COVID-19: a systematic review. Invest Educ Enferm 2020; 38 (2): e13. doi: 10.17533/udea.iee.v38n2e13.
35. Balazy A, Toivola M, Adhikari A, Sivasubramani SK, Reponen T, Grinshpun SA. Do N95 respirators provide 95% protection level against airborne viruses, and how adequate are surgical masks? Am J Infect Control 2006; 34 (2): 51-57. doi: 10.1016/j.ajic.2005.08.018.
36. Weber A, Willeke K, Marchioni R, Myojo T, McKay R, Donnelly J, Liebhaber F. Aerosol penetration and leakage characteristics of masks used in the health care industry. Am J Infect Control 1993; 21 (4): 167-173. doi: 10.1016/0196-6553(93)90027-2.
37. FDA (US Food and Drug Administration). N95 Respirators, surgical masks, face masks, and barrier face coverings. Content current as of: 3 March 2020 [cited 2022, May 20]. Available from: web.archive.org/web/20200305191726/https://www.fda.gov/medical-devices/personal-protective-equipment-infection-control/n95-respirators-and-surgical-masks-face-masks
38. FDA (US Food and Drug Administration). N95 Respirators, surgical masks, face masks, and barrier face coverings. Content current as of: 19 July 2022 [cited 2022, July 20]. Available from: www.fda.gov/medical-devices/personal-protective-equipment-infection-control/n95-respirators-surgical-masks-face-masks-and-barrier-face-coverings
39. Smith JD, MacDougall CC, Johnstone J, Copes RA, Schwartz B, Garber GE. Effectiveness of N95 respirators versus surgical masks in protecting health care workers from acute respiratory infection: a systematic review and meta-analysis. CMAJ 2016; 188 (8): 567-574. doi: 10.1503/cmaj.150835.
40. Lee SA, Grinshpun SA, Reponen T. Respiratory performance offered by N95 respirators and surgical masks: human subject evaluation with NaCl aerosol representing bacterial and viral particle size range. Ann Occup Hyg 2008; 52 (3): 177-185. doi: 10.1093/annhyg/men005.
41. Radonovich LJ Jr, Simberkoff MS, Bessesen MT, Brown AC, Cummings DAT, Gaydos CA, Los JG, Krosche AE, Gibert CL, Gorse GJ, Nyquist AC, Reich NG, Rodriguez-Barradas MC, Price CS, Perl TM; ResPECT investigators. N95 Respirators vs medical masks for preventing influenza among health care personnel: a randomized clinical trial. JAMA 2019; 322 (9): 824-833. doi: 10.1001/jama.2019.11645.
42. Zanotti B, Parodi PC, Riccio M, De Francesco F, Zingaretti N. Can the elastic of surgical face masks stimulate ear protrusion in children? Aesthetic Plast Surg 2020; 44 (5): 1947-1950. doi: 10.1007/s00266-020-01833-9.
43. Levine JM, Ayello EA, Persaud B, Spinner R. Medical device-related pressure Injury to the ear from a mask. Adv Skin Wound Care 2021; 34 (7): 380-383. doi: 10.1097/01.ASW.0000752712.31139.c0.
44. Gattani VN, Gattani G. 'Novel' ear injuries in novel corona virus era. J Maxillofac Oral Surg 2022; 21 (1): 112-114. doi: 10.1007/s12663-021-01577-2.
45. Lazzarino AI, Steptoe A, Hamer M, Michie S. Covid-19: Important potential side effects of wearing face masks that we should bear in mind. BMJ. 2020 May 21;369:m2003. doi: 10.1136/bmj.m2003. Comment of: Greenhalgh T, Schmid MB, Czypionka T, Bassler D, Gruer L. Face masks for the public during the Covid-19 crisis. BMJ 2020; 369: m1435. doi: 10.1136/bmj.m1435.
46. Moore JJ. Malattie terribili e atroci cure. La storia della medicina attraverso i secoli. Logos, Modena, 2020.
47. Kwok YL, Gralton J, McLaws ML. Face touching: a frequent habit that has implications for hand hygiene. Am J Infect Control 2015; 43 (2): 112-114. doi: 10.1016/j.ajic.2014.10.015.

48. Schönhofer B, Rosenblüh J, Kemper P, Voshaar T, Köhler D. Einfluss des Mundschutzes auf die Atemarbeit bei Patienten mit chronischer Belastung der Atempumpe [Effect of a face mask on work of breathing in patients with chronic obstructive respiratory disease]. Pneumologie 1995; 49 (Suppl. 1): 209-211.

49. Yip WL, Leung LP, Lau PF, Tong HK. The effect of wearing a face mask on body temperature. Hong Kong J Emerg Med 2005; 12 (1): 23-27. doi: 10.1177/102490790501200102.

50. Li Y, Tokura H, Guo YP, Wong AS, Wong T, Chung J, Newton E. Effects of wearing N95 and surgical facemasks on heart rate, thermal stress and subjective sensations. Int Arch Occup Environ Health 2005; 78 (6): 501-509. doi: 10.1007/s00420-004-0584-4.

51. Harrison MR, Hysing ES, Bo G. Control of body temperature: use of the respiratory tract as a heat exchanger. J Pediatr Surg 1977; 12 (6): 821-828. doi: 10.1016/0022-3468(77)90590-5.

52. Childs C. Human brain temperature: regulation, measurement and relationship with cerebral trauma: part 1. Br J Neurosurg 2008; 22 (4): 486-496. doi: 10.1080/02688690802245541.

53. The Simpsons. The Fool Monty (November 21, 2010) [cited 2022, May 20]. Available from: www.youtube.com/watch?v=Yjw1z6fsxA8

54. Valli AM. Virus e Leviatano. Liberilibri, Macerata, 2020.

55. Presidente della Repubblica. Decreto Legislativo 9 aprile 2008, n. 81: Attuazione dell'articolo 1 della legge 3 agosto 2007, n. 123, in materia di tutela della salute e della sicurezza nei luoghi di lavoro. GU Serie Generale n.101 del 30-04-2008 - Suppl. Ordinario n. 108 [cited 2022, May 20]. Available from: www.gazzettaufficiale.it/eli/id/2008/04/30/008G0104/sg

56. Direzione Generale della Prevenzione Sanitaria. Microclima e benessere termico. Content current as of: 16 dicembre 2015 [cited 2022, May 20]. Available from: www.salute.gov.it/portale/temi/p2_6.jsp?lingua=italiano&id=4387&area=indor&menu=vuoto

57. Ministero dell'Istruzione, Ufficio Scolastico Regionale per il Veneto, Direzione Generale. Piano per la ripartenza 2020/2021. Manuale Operativo [cited 2022, May 20]. Available from: //istruzioneveneto.gov.it/wp-content/uploads/2020/07/Manuale-operativo-USRV-definitivo-7-7-2020.pdf

58. Analitis A, Katsouyanni K, Biggeri A, Baccini M, Forsberg B, Bisanti L, Kirchmayer U, Ballester F, Cadum E, Goodman PG, Hojs A, Sunyer J, Tiittanen P, Michelozzi P. Effects of cold weather on mortality: results from 15 European cities within the PHEWE project. Am J Epidemiol 2008; 168 (12): 1397-1408. doi: 10.1093/ aje/kwn266.

59. Mäkinen TM, Palinkas LA, Reeves DL, Pääkkönen T, Rintamäki H, Leppäluoto J, Hassi J. Effect of repeated exposures to cold on cognitive performance in humans. Physiol Behav 2006; 87 (1): 166-176. doi: 10.1016/j.physbeh.2005.09.015.

60. Pievani T. L'assassinio di un diverso. Le Scienze 2021; 638: 15.

61. Moore JJ. Pistole, pugnali e veleni. Delitti illustri ed assassini efferati. Logos, Modena, 2019.

62. Adichie CN. Il pericolo di un'unica storia. Giulio Einaudi Editore, Torino, 2009.

63. Speranza.R. Perché guariremo. Dai giorni più duri a una nuova idea di salute. Feltrinelli, Milano, 2020.

64. Ciccolella C, Valesini G. La grande inchiesta di Report sulla pandemia. Chirelettere, Milano, 2021.

65. Zambon F. Il pesce piccolo. Una storia di virus e segreti. Feltrinelli; Milano, 2021.

66. Desiderio G. L'individualismo statalista. La vera religione degli italiani. Lirilibri, Macerata, 2017.

67. Zanotti B, Vecchio A., Consoli D. COVID-19: il valore della prospettiva asimmetrica. Progress in Neuroscience 2020; 1-4 (Suppl. 1): 5-8.

68. Quay S. Un'analisi bayesiana del COVID-19: le sue origini viste da un "allibratore". In P. Bernard, Dalgleish A, Quay S (editors): L'origine del virus. L'origine del virus. Le verità tenute nascoste che hanno ucciso milioni di persone. Chiarelettere, Milano, 2021: 97-133.

69. Dalgleish A. Un camaleonte chimerico. In P. Bernard, Dalgleish A, Quay S (editors): L'origine del virus. L'origine del virus. Le verità tenute nascoste che hanno ucciso milioni di persone. Chiarelettere, Milano, 2021: 97-133.

70. Nataloni A. Zanotti B. La chiamarono "Spagnola": ma venne dal mare. Un promemoria che non andava dimenticato. Progress in Neuroscience 2020; 1-4 (Suppl. 1): 23-30.

71. Breccia G., Frediani A. Epidemie e guerre che hanno cambiato il corso della storia. Newton Compton, Rome, 2021.

72. Folisi E. La Spagnola. La tragica epidemia influenzale del '900. Gasperi,Udine, 2020.

73. Spinney L. 1918. L'influenza spagnola. La pandemia che cambiò il mondo. Marsilio, Venezia, 2018.

74. Moore JJ. Malattie terribili e atroci cure. La storia della medicina attraverso i secoli. Logos, Modena, 2020.

75. Desiderio G. Teoria generale delle stronzate. Come si distrugge una nazione. Castelvecchi, Rome, 2021.

76. Borgonovo F., Conti C., Rossitto A. Epidemia di balle. La Verità, Milan, 2021.

77. Chomsky N. Media e potere. Bepress, Lecce, 2014.

77. Rubin M. The boiled frog syndrome. Alyson Publications, New York (US), 1987.

79. Quinn D. The story of B. Bantam Dell, New York (US), 1996.

80. Alighieri Dante. La divina commedia. Biblioteca Universale Rizzoli, Milano, 2007.

Original article

□ Microsurgical removal of symptomatic vestibular schwannomas in the eighth decade of life

L. MASTRONARDI*✧, A. CAMPIONE*, E. CARPINETA*, R. SUFIANOV**✧,
F. BOCCACCI*, A. KOVALEV**, R. ROPERTO*, A SUFIANOV**✧

* *Department of Neurosurgery, "S. Filippo Neri" Hospital, ASL Roma 1, Rome, Italy*
** *Federal Centre of Neurosurgery, Tyumen, Russian Federation*
✧ *Department of Neurosurgery, The State Education Institution of Higher Professional Training, The First Sechenov Moscow State Medical University under Ministry of Health, Russian Federation*

***SUMMARY:* Aims**: *Older adults constitute an ever-growing cohort among patients affected by vestibular schwannoma. We conducted a retrospective analysis of clinical presentation and surgical outcomes of 30 patients aged ≥ 70, operated on in two neurosurgical centers for unilateral vestibular schwannoma.*
Materials and Methods: *American Society of Anesthesiology (ASA) grade I-II patients and grade III ones with life-threatening tumors were enrolled. The House-Brackmann (HB) scale was used to assess facial nerve postoperative functional outcome. Tumor size was categorized according to Koos' classification. A retrosigmoid approach was used in all the surgical operations. Extent of removal was described as total, near total (> 95%), subtotal (> 90%).*
Results: *Mean age within the cohort was 74 years; 53% ASA II, 47% ASA III. Mean tumor size was 2.7cm (range 1.5-4.2 cm). Gross-total or near-total resection was achieved in 80% of cases, subtotal totaling 20% of cases. Perioperative mortality - i.e., within one month of the operation - was zero. Long-term facial outcome at six months: HB I 50%, HB II 27%, HB III 23%. Transient complications occurred only in case of large tumors. The occurrence of remnant growth after subtotal resection was observed in 3 cases, but a second surgery was never deemed necessary.*
Conclusions: *In patients in good general conditions, age does not appear to be a major contraindication for microsurgical resection of vestibular schwannomas. Long-term facial nerve results are satisfactory in most patients in the eighth decade of life and complications are the same as observed among youngsters. Factors associated with higher complication rates are poor preoperative general condition (ASA III) and large tumors (Koos IV), especially when treated with surgical gross total resection.*

KEY WORDS: *Age, Facial nerve, Facial nerve position, Facial nerve preservation, Older adults, Retrosigmoid approach, Vestibular schwannoma.*

□ INTRODUCTION

Vestibular schwannoma is the most common tumor of the cerebello-pontine angle, ranging from 85 to 92% of tumors[3,15,25,26,33]. The clinical presentation correlates with the structures gradually compressed by the tumor. Over the past 40 years, the incidence of VS has increased steadily more than 10 times and the mean

Corrispondence: Prof. Luciano Mastronardi, Department of Neurosurgery, "S. Filippo Neri" Hospital, via Giovanni Martinotti 20, 00135 Roma (RM), e-mail: luciano.mastronardi@aslroma1.it
Progress in Neurosciences 2021; 6 (1-4): 19-26. *ISSN: 2240-5127.*

LIST OF ACRONYMS AND ABBREVIATIONS: **AAO-HNS** = American Academy of Otolaryngology - Head and Neck Surgery; **ABR** = Auditory Brainstem Responses; **ASA** = American Society of Anesthesiologists physical status classification system; **CSF** = CerebroSpinal Fluid; **CT** = Computed Tomography; **FN** = Facial Nerve; **GTR** = Gross Total Resection; **HB** = House-Brackmann; **HL** = Hearing Level; **i.e.** = id est (Latin for that is); **MRI** = Magnetic Resonance Imaging; **MS** = MicroSurgery; **NTR** = Near Total Resection; **RT** = Radiotherapy; **SDS** = Speech Discrimination Score; **SRS** = Stereotactic RadioSurgery; **STR** = SubTotal Resection; **VS** = Vestibular Schwannoma; **WW** = Watchful Waiting.

age at diagnosis has increased from approximately 49 to 60 years(2,5,17,21,22). The treatment of VS depends on the characteristics of both the patient and the tumor. Tumor size and growth pattern as well as patient age, symptoms and co-morbidities determine the treatment of choice among three main options: conservative therapy with watchful waiting, stereotactic radiosurgery, and microsurgery(25,26,33). The goal of modern management of VS is to improve quality of life and preserve neurological function while maintaining low mortality and morbidity rates.

Aims. In this study, we retrospectively analyzed the clinical and surgical data of a consecutive series of 30 symptomatic patients aged 70 or older with sporadic unilateral VS, operated at two different neurosurgical centers. We evaluated surgical outcome while highlighting the extent of tumor removal, postoperative nerve functions and complications.

□ MATERIALS AND METHODS

■ **Patient population.** We retrospectively reviewed a series of 30 consecutive cases of vestibular schwannoma in patients aged 70 or older (eighth decade of life) who were operated on at two neurosurgical centers: the Neurosurgical Division of "San Filippo Neri" Hospital in Rome and the Federal Centre of Neurosurgery in Tyumen, between September 2010 and April 2019. General health conditions were graded according to the ASA score: only patients assessed as grades I, II and III were considered for surgery; all ASA grade III patients were affected by large and life-threatening VS.

Only patients with sporadic VS were included in the study. Clinical data such as patients' age, sex, presenting symptoms, symptoms duration and tumor size were recorded. Preoperative neuroimaging studies included temporal bone CT and gadolinium-enhanced MRI for all patients.

Tumor size was measured according to the international standard - i.e. the largest extrameatal tumor diameter on post-contrast axial MRI(11). Large tumors were considered those with a maximal diameter ≥ 30 mm in the cerebellopontine angle, as opposed to small tumors with a maximal diameter < 30 mm. According to Koos et al.(12), tumors are classified into four grades in relation to maximal diameter and relation with contiguous anatomical structures.

Microsurgery via a retrosigmoid approach was performed in all cases.

The course of the FN was classified into four different patterns according to its position: anterior (or ventral) to tumor surface, anterior-inferior (or ventral-inferior), anterior-superior (or ventral-superior), and dorsal(14,24).

The preoperative audio-vestibular evaluation included pure tone audiometry, SDS assessment, caloric test and ABR. Hearing level was determined according to the AAO-HNS classification(1): Class A (SDS > 70%) and B (SDS > 50%) were categorized as bearing good hearing, while useful hearing preservation included Classes A, B and C (SDS < 50%). The location of the FN and its adherence to the tumor were evaluated by reviewing the radiological images and the surgical video records. FN function was assessed according to the HB grading system(8) and was evaluated preoperatively, at discharge and at the final follow-up (minimum 6 months). HB grades I and II were considered as a favorable outcome while grades III, IV and V were unfavorable. Other possible complications such as postoperative hemorrhage, CSF leak, infection, neurological defects of new onset and perioperative mortality rate - i.e. within one month of the operation - were recorded.

We graded the extent of tumor removal as gross total resection, near total (linear millimetric tumor remnant), subtotal (residual tumor volume < 10%) and partial (residual tumor volume > 10%). The extent of resection was evaluated on postoperative MRIs through a blind review by one member of our neurosurgical staff (E.C.) and a neuroradiologist. The blind evaluation was then compared with the impression of the surgeon as written in the operative report.

■ **Facial nerve intaoperative neuromonitoring.** The intraoperative nerve monitoring system (Nimbus i-Care 100 intraoperative neurophysiologic monitoring; Newmedic Division of Hemodia, Labège, France), with electrodes inserted in orbicularis oris and orbicularis oculi muscles, was routinely used intraoperatively to detect FN responsivity.

■ **Follow-up.** The long-term FN outcome evaluation was performed at six months postoperatively and assessed according to HB scale. Clinical and radiological follow-up was scheduled at six months postoperatively and then once a year. The follow-up period ranged between 12 and 115 months (mean 63; median 63.5).

☐ RESULTS

Thirty patients underwent microsurgical removal via a retrosigmoid approach for unilateral VS. Patient age ranged from 70 to 80 years old, with a mean age of 74 years. There were 13 female (43.3%) and 17 male (56.7%) patients. ASA score II was assigned in 16 cases (53%) and ASA III in 14 (47%). In 19 cases (63.3%) the tumor was left-sided, while in 11 cases it was on the right side. Major clinical symptoms included progressive sensorineural hearing loss, gait disturbance and facial paresthesia. The mean tumor size was 2.7 cm (range 1.5-4.2 cm). In 22 cases (73.3%), the maximum tumor diameter was ≥ 3 cm (Table 1).

The preoperative auditory assessment showed that 19 patients (63.3%) suffered from grade D hearing loss, 9 patients (30%) suffered from grade C hearing impairment, while 2 patients (6.7%) were assessed as grade B. According to the HB scale, preoperative HB IV FN palsy was present in 4 cases.

GTR or NTR was achieved in 24 cases (80%) and STR in 6 cases (20%). The cisternal segment of the FN was anterosuperior to the tumor in 14 (46.7%) cases, anterior in 10 (33.3%), and anteroinferior in 6 (20%). The follow-up ranging from 12 to 111 months demonstrated that a second surgery for re-growth of residual tumor was never necessary due to tumor remnant stability.

Hearing preservation was achieved in 1 patient out of two possible candidates (class B of AAO-HNS grading system). As shown in Table 2, excluding 4 cases with preoperative HB IV before and after surgery, at the final follow up (April 30, 2020), 13 out (50%), 8 (27%), and 5 (23%) patients showed a FN function of HB I, II and III, respectively. The 5 patients with a postoperative HB III FN result had a tumor with a maximum diameter larger than 3 cm (Koos IV): GTR or NTR was achieved in 4 cases, while STR removal in the remaining 2 cases.

The mortality rate was zero. CSF leak, wound infection and postoperative hydrocephalus were not observed. Postoperative transient complications were

Vestibular schwannoma in patients aged ≥ 70 years		
Sex	Female	13 (*43.3%*)
	Male	17 (*56.7%*)
Mean age ± SD (years)	74 ± 3.8 (range: 70-80)	
Side	Left	19 (*63.3%*)
	Right	11 (*36.7%*)
Mean diameter (cm)	2.7 (range: 1.5-4.2)	
ASA score	II	16 (*53%*)
	III	14 (*47%*)
Koos grade	II	6 (*20%*)
	III	12 (*40%*)
	IV	12 (*40%*)
AAO-HNS hearing function	B	2 (*6.7%*)
	C	9 (*30%*)
	D	19 (*63.3%*)
Preoperative HB grade	I	26 (*86.7%*)
	IV	4 (*13.3%*)

Table 1. Preoperative clinical and radiological characteristics of the study population. *Legend:* AAO-HNS = American Academy of Otolaryngology - Head and Neck Surgery; HB = House-Brackmann; SD = Standard Deviation.

Facial nerve outcome and postoperative complications		
FN outcome at 6 months	HB I	13/26 (*50.0%*)
	HB II	8/26 (*30.8%*)
	HB III	5/26 (*19.2%*)
	HB IV	4 (*stable as preoperative*)
Mortality	null	
Permanent complications	null	
Transient complications (observed only in Koos IV - ASA II VS)	Imbalance	10 (*33.3%*)
	Dizziness	9 (*30.0%*)
	Hemiparesis	4 (*13.3%*)
	Diplopia (abducens nerve palsy, recovered after three months)	1 (*3.3%*)
Second surgery for growing tumor remnant	null	

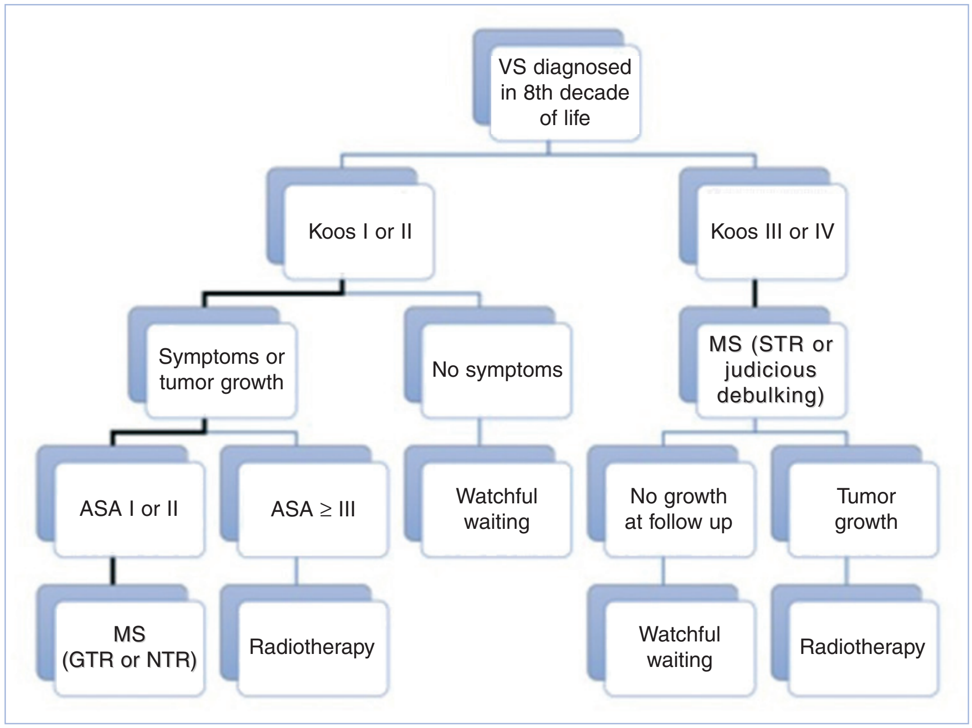
Table 2. Facial nerve outcome and postoperative complications. *Legend:* ASA = American Society of Anesthesiologists; FN = Facial Nerve; HB = House-Brackmann; VS = Vestibular Schwannoma.

present only in patients with Koos IV VS and ASA III: imbalance in 10 cases (33.3%), dizziness in 9 (30%), hemiparesis in 4 (13.3%), and diplopia in one case (3.3%), which was caused by abducens nerve paralysis and lasted 3 months.
A second operation for recurrence was not performed on any patient.
Re-growth of residual tumor after STR removal was observed in 3 cases, 2 treated with SRS and one observed with seriated MRI scans.

☐ DISCUSSION

Older population is currently increasing around the world. Diffusion and development of MRI have allowed for the incidental and intentional diagnosis of VS in patients aged 70 years old or older.
Decision-making regarding surgery in the elderly population is never easy and in certain cases represents a challenge, as several co-morbidities and short life expectancy should be balanced against the surgical benefit in this group of patients. On the other hand, most elders have a better life expectancy and quality of life than in the past. Additionally, surgery is safe, and postoperative intensive care improves patient recovery(6,16,18-20,23,27,31). Consequently, the surgeon is called to perform surgical treatment for older adults ever more frequently.
Treatment options for elders are the same as those used among youngsters: "wait and watch" management; microsurgery and stereotactic radiosurgery.
Patients with small-sized VS (Koos I and II grades) in their eighth decade of life, without contact with the brainstem, tumor growth or disabling symptoms must be managed conservatively and followed up with contrast-enhanced MRI first at 6 months-1 year from diagnosis(16,18). Tumor growth may be extremely variable, from no growth to a few millimeters per year. In other cases, it is possible to observe tremendous neoplasm enlargement with brainstem compression and severe neurological impairment. Nuseir et al.(16) reported a mean growth rate of 2 mm per year. Perry et al.(18) in a series of 41 older patients affected by VS, observed tumor growth in 51.2% during a median follow-up of 24 months, with a growth rate of 3.2 mm per year. On the other hand, Roehm and Gantz(23) reported a median tumor growth per year of 1,4 mm in 114 VS.
In a series of 232 VS, Nuseir et al.(16) suggested microsurgery in elderly patients with small-sized tumors (Koos I-II) associated with severe persistent vertigo or in case of rapid growth observed during WW. Microsurgery was also indicated in patients with large-sized tumor (Koos III-IV) compressing the brainstem(16).
Roehm and Gantz(23), in their series of 270 patients aged over 65 affected by VS, decided for MS based on: tumor size (diameter > 2.5), tumor growth, symptoms (most commonly vertigo or imbalance), the pos-

Figure 1. Treatment algorithm for vestibular schwannoma in the eighth decade of life. The paths leading to surgery are emphasized to clarify the main indications for microsurgical removal. *Legend*: ASA = American Society of Anesthesiologists; GTR = Gross Total Resection; MS = MicroSurgery; NTR = Near Total Resection; STR = SubTotal Resection; VS = Vestibular Schwannoma.



sibility of attempting hearing preservation, brainstem contact or compression, and patient request[23].
In our series of 30 elderly patients (mean age > 70 years), we used an algorithm inspired by the clinical reasoning of previously cited Authors, for decision-making (Figure 1).
Tumor features that prevented the surgeon from attempting GTR was Koos grade III or IV or cystic tumors, especially in patients with preoperative ASA grade III. Microsurgery aiming at GTR or NTR is recommended in symptomatic or growing Koos I or II VS in elderly patients with a preoperative satisfactory physical status. For cases of Koos III or IV VS, brainstem compression symptoms themselves generally compromise a patient's physical status so that the ASA score cannot be a fixed parameter for decision-making for a surgical procedure. Instead, microsurgery should aim at relieving symptoms and decompressing the brainstem while preserving neurological functions. While evaluating the surgical benefits and risks, in these cases we preferred STR with a goal of careful debulking. Conversely, other studies recommended SRS for patients with a poor general condition or after subtotal or partial tumor resection[16,31]. Some other Authors[28,32] proposed radiotherapy (gamma-knife, proton beam, and fractionated SRS) as a substitute for WW, for small and medium-sized VS, to counteract the unpredictability of tumor growth in such instances. Indeed, Shirato et al.[28] reported a growth rate of 0.75 mm per year in RT-treated patients versus a growth rate of 3.87 mm per year in the observational control group.
According to Samii and Matthies[25], the hearing preservation rate in the irradiated group was similar to that within the control group treated surgically. Considering these findings and the -though rare- possibility of eventual malignant transformation of VS, it has been argued that SRS should not be the treatment of choice in small and medium-sized growing and symptomatic tumors[18].
In elderly patients treated with elective STR, tumor remnant growth rate is higher than that observed among younger patients (38% versus 19%)[31]. Considering these findings, several Authors[16,31] recommend adjuvant RT after STR among elders. Our standpoint regarding SRS is to reserve such treatment in case of post-operative remnant progression after STR and after adequate postoperative neuroradiological follow-up. SRS may play a role as a primary treatment option in cases of Koos I-II VS affecting severely compromised patients (ASA III-IV).
The outcome of elderly patients affected from VS treated with MS has been described by several Authors.

Samii et al.[27], in 1992, first reported factors predictive of postoperative outcome in a series of 61 older adults operated on by a retrosigmoid approach. Their results showed that three factors seemed to influence the outcome: preoperative ASA score; preoperative Karnofsky score, and tumor size.

Sylvester et al.[29] performed a retrospective analysis of the National Inpatient Sample for patients undergoing VS surgery that included 4,137 people. They compared the in–hospital complication rate between older and younger patients. Elders had a longer hospital stay (6.5 *versus* 5.4 days) but not greater hospital charges. The elderly cohort showed a significantly higher postoperative rate of transitory complications consisting of cerebellar signs or consequences of prolonged immobilization in bed - i.e., deep venous thrombosis, pneumonia. FN palsy, other cranial nerve deficits, and CSF leak did not have a greater incidence among the elders. The in-hospital mortality rate among the elderly population was greater than among younger patients, and even if quite low, was in line with other Authors[16,18,31]. In our series, a poor postoperative course was associated with poor preoperative physical status rather than the age or surgical procedure, per se. Furthermore, after stratification based on the extent of tumor resection, complication rates among cases of GTR were higher than in cases of NTR or STR. Therefore, STR or debulking was planned preoperatively in most cases of large tumors, especially in ASA III patients.

Some Authors reported worse FN outcomes in the elderly population as compared to younger patients[7,16, 18,31]. They predicted that unfavorable FN results were due to a decreased tissue regeneration ability caused by atherosclerotic disease in vasa nervorum vessels[16,18,31].

Another complication observed in the elderly population is postoperative imbalance. In our series, it was observed in one-third of patients after surgery. The vestibular system has a less functional reserve in older patients and consequently struggles with attempts to compensate for both tumor-induced and/or iatrogenic damages[10]. In addition, the elderls have comorbidities that impact their balance function; in particular, less visual acuity diminishes proprioception and cerebellar functions[31].

However, rehabilitation therapy reinforces the central compensatory mechanisms that account for a better vestibular outcome in case of peripheral vestibular damage. Although no randomized clinical trials are available in this field regarding the elderly population[13], vestibular rehabilitation seems to normalize balance function even in patients up to 99 years[4]. Intensive pre- and postoperative vestibular training featuring visual biofeedback is an instrument in accelerating recovery after VS microsurgery[9,30]. In such context, the administration of preoperative intratympanic gentamicin as a means of prehabilitation has demonstrated efficacy in terms of preoperative vestibular deafferentation, which lets the patient elaborate compensatory mechanisms even before surgery[30]. Elderly patients have a good chance of benefiting from VS surgery in terms of quality of life and dizziness/vertigo resolution. Therefore, surgical treatment should not be precluded based only on age per se.

☐ CONCLUSIONS

Based on our limited experience and that of the international literature, age itself does not seem to represent a major contraindication against microsurgical removal of VS, especially in patients in good general condition. Long-term FN results are satisfactory in most patients in the eighth decade of life and complications are the same as observed among youngsters. Factors associated with higher complication rates are poor preoperative general condition (ASA III) and large tumors (Koos IV), especially when treated with surgical GTR.

☐ REFERENCES

1. Committee on Hearing and Equilibrium guidelines for the evaluation of hearing preservation in acoustic neuroma (vestibular schwannoma). American Academy of Otolaryngology-Head and Neck Surgery Foundation, INC. Otolaryngol Head Neck Surg 1995; 113 (3): 179-180. doi: 10.1016/S0194-5998(95)70101-X
2. Chan SA, Marinelli JP, Hahs-Vaughn DL, Nye C, Link MJ, Carlson ML. Evolution in management trends of sporadic vestibular schwannoma in the United States over the last half-century. Otol Neurotol 2021; 42 (2): 300-305. doi: 10.1097/MAO.0000000000002891
3. Dang L, Tu NC, Chan EY. Current imaging tools for vestibular schwannoma. Curr Opin Otolaryngol Head Neck Surg 2020; 28 (5): 302-307. doi: 10.1097/MOO.0000000000000647
4. Deems DA, Deems RO, O'Malley BW Jr. Managing challenges in an aging vestibular system: rehabilitation strategies normalize balance function in a cohort of patients up to 99 years. Ear Nose Throat J 2019; 98 (1): 37-43. doi: 10.1177/0145561318824541

5. Gal TJ, Shinn J, Huang B. Current epidemiology and management trends in acoustic neuroma. Otolaryngol Head Neck Surg 2010; 142 (5): 677-681. doi: 10.1016/j.otohns.2010.01.037
6. Glasscock ME 3rd, Pappas DG Jr., Manolidis S, Von Doersten PG, Jackson CG, Storper IS. Management of acoustic neuroma in the elderly population. Am J Otol Mar 1997;18 (2): 236-241.
7. Helal A, Graffeo CS, Perry A et al. Differential impact of advanced age on clinical outcomes after vestibular schwannoma resection in the very elderly: cohort study. Oper Neurosurg 2021; 21 (3): 104-110. doi: 10.1093/ons/opab170
8. House JW, Brackmann DE. Facial nerve grading system. Otolaryngol Head Neck Surg 1985; 93 (2): 146-147. doi: 10.1177/019459988509300202
9. Hruba S, Chovanec M, Cada Z et al. The evaluation of vestibular compensation by vestibular rehabilitation and prehabilitation in short-term postsurgical period in patients following surgical treatment of vestibular schwannoma. Eur Arch Otorhinolaryngol 2019; 276 (10): 2681-2689. doi: 10.1007/s00405-019-05503-8
10. Jahn K. The aging vestibular system: dizziness and imbalance in the elderly. Adv Otorhinolaryngol 2019; 82: 143-149. doi: 10.1159/000490283
11. Kanzaki J, Tos M, Sanna M, Moffat DA, Monsell EM, Berliner KI. New and modified reporting systems from the consensus meeting on systems for reporting results in vestibular schwannoma. Otol Neurotol 2003; 24 (4): 642-648. doi: 10.1097/00129492-200307000-00019
12. Koos WT, Day JD, Matula C, Levy DI. Neurotopographic considerations in the microsurgical treatment of small acoustic neurinomas. J Neurosurg 1998; 88 (3): 506-512. doi: 10.3171/jns.1998.88.3.0506
13. Martins ESDC, Bastos VH, de Oliveira Sanchez M et al. Effects of vestibular rehabilitation in the elderly: a systematic review. Aging Clin Exp Res 2016; 28 (4): 599-606. doi: 10.1007/s40520-015-0479-0
14. Mastronardi L, Cacciotti G, Roperto R, Di Scipio E, Tonelli MP, Carpineta E. Position and course of facial nerve and postoperative facial nerve results in vestibular schwannoma microsurgery. World Neurosurgery 2016; 94: 174-180. doi: 10.1016/j.wneu.2016.06.107
15. Matthies C, Samii M, Krebs S. Management of vestibular schwannomas (acoustic neuromas): radiological features in 202 cases - their value for diagnosis and their predictive importance. Neurosurgery 1997; 40 (3): 469-481. doi: 10.1097/00006123-199703000-00009
16. Nuseir A, Sequino G, De Donato G, Taibah A, Sanna M. Surgical management of vestibular schwannoma in elderly patients. Eur Arch Otorhinolaryngol 2012; 269 (1): 17-23. doi: 10.1007/s00405-011-1566-2
17. Pandrangi VC, Han AY, Alonso JE, Peng KA, St John MA. An Update on Epidemiology and Management Trends of Vestibular Schwannomas. Otol Neurotol 2020; 41 (3): 411-417. doi: 10.1097/MAO.0000000000002542
18. Perry BP, Gantz BJ, Rubinstein JT. Acoustic neuromas in the elderly. Otol Neurotol 2001; 22 (3): 389-391. doi: 10.1097/00129492-200105000-00020
19. Pulec JL. Acoustic neuroma surgery in geriatric patients. Ear Nose Throat J 1999; 78 (6): 429-430, 433-436, 438-440 passim.
20. Pulec JL, Giannotta SL. Acoustic neuroma surgery in patients over 65 years of age. Ear Nose Throat J 1995; 74 (1): 21-27.
21. Reznitsky M, Petersen M, West N, Stangerup SE, Caye-Thomasen P. Epidemiology of vestibular schwannomas - prospective 40-year data from an unselected national cohort. Clin Epidemiol 2019; 11: 981-986. doi: 10.2147/CLEP.S218670
22. Reznitsky M, Petersen M, West N, Stangerup SE, Caye-Thomasen P. The natural history of vestibular schwannoma growth-prospective 40-year data from an unselected national cohort. Neuro Oncol 2021; 23 (5): 827-836. doi: 10.1093/neuonc/noaa230
23. Roehm PC, Gantz BJ. Management of acoustic neuromas in patients 65 years or older. Otol Neurotol 2007; 28 (5): 708-714. doi: 10.1097/01.mao.0000281805.44197.ec
24. Sameshima T, Morita A, Tanikawa R, et al. Evaluation of variation in the course of the facial nerve, nerve adhesion to tumors, and postoperative facial palsy in acoustic neuroma. J Neurol Surg B Skull Base 2013; 74 (1): 39-43. doi: 10.1055/s-0032-1329625
25. Samii M, Matthies C. Management of 1000 vestibular schwannomas (acoustic neuromas): surgical management and results with an emphasis on complications and how to avoid them. Neurosurgery 1997; 40 (1): 11-21. doi: 10.1097/00006123-199701000-00002
26. Samii M, Matthies C. Management of 1000 vestibular schwannomas (acoustic neuromas): the facial nerve - preservation and restitution of function. Neurosurgery 1997; 40 (4): 684-694. doi: 10.1097/00006123-199704000-00006
27. Samii M, Tatagiba M, Matthies C. Acoustic neurinoma in the elderly: factors predictive of postoperative outcome. Neurosurgery 1992; 31 (4): 615-619. doi: 10.1227/00006123-199210000-00001
28. Shirato H, Sakamoto T, Sawamura Y et al. Comparison between observation policy and fractionated stereotactic radiotherapy (SRT) as an initial management for vestibular schwannoma. Int J Radiat Oncol Biol Phys 1999; 44 (3): 545-550. doi: 10.1016/s0360-3016(99)00079-6
29. Sylvester MJ, Shastri DN, Patel VM, et al. Outcomes of vestibular schwannoma surgery among the elderly. Otolaryngol Head Neck Surg 2017; 156 (1): 166-172. doi: 10.1177/0194599816677522
30. Tjernstrom F, Fransson PA, Kahlon B, et al. PREHAB vs. REHAB - presurgical treatment in vestibular schwannoma surgery enhances recovery of postural control better than postoperative rehabilitation: Retrospective case series. J Vestib Res 2018; 27 (5-6): 313-325. doi: 10.3233/VES-170626

31. Van Abel KM, Carlson ML, Driscoll CL, Neff BA, Link MJ. Vestibular schwannoma surgery in the elderly: a matched cohort study. J Neurosurg 2014; 120 (1): 207-217. doi: 10.3171/2013.6.JNS122433

32. van Roijen L, Nijs HG, Avezaat CJ et al. Costs and effects of microsurgery versus radiosurgery in treating acoustic neuroma. Acta Neurochir 1997; 139 (10): 942-948. doi: 10.1007/BF01411303

33. Wu H, Zhang L, Han D, et al. Summary and consensus in 7th International Conference on acoustic neuroma: An update for the management of sporadic acoustic neuromas. World J Otorhinolaryngol Head Neck Surg 2016; 2 (4): 234-239. doi: 10.1016/j.wjorl.2016.10.002

Acknowledgements. *We thank Ms Lori Radcliffe, BSN, RN for assistance with English language editing.*

Ethics Statement. *The study involves human participants: for this reason, it has been reviewed and approved by local Ethics committee (Institutional Review Board) of the Hospital (Lazio 1, ASL Roma 1). A written consent for scientific treatment of personal data was obtained from any patient before surgery. No potentially identifiable human images or data are presented in this study.*

Funding. *This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.*

Disclosure. *The Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.*

Original article

□ Efficacy and safety of the magnesium-hydroxyapatite bone graft substitute in postero-lateral spinal fusion: observational, spontaneous clinical study

B. ZANOTTI*, A. NATALONI**, V. CANELLA**, F. MUGGIOLU*

* *Neurosurgery Unit, Neuroscience Department, "C. Poma" Hospital, Mantua, Italy*
** *Finceramica S.p.A., Faenza (Ravenna), Italy*

SUMMARY: ***Aims.*** *Iliac Crest Bone Graft (ICBG) is considered the "gold standard" option for achieving fusion in spinal surgical procedures, thanks to its known osteoinductive and osteoconductive properties. However, complications related to harvesting and donor site morbidity have been largely reported in literature, favouring the development of a wide range of alternative products to be used as bone graft extenders or substitutes. Ceramic-based biomaterials are since years employed as bone graft substitutes to replace autograft, due to their similarity with the mineral component of human bone. Recently, new generation of hydroxyapatite biomaterials have been developed with superior properties to promote a faster remodelling and new formation of human bone, and a rapid cell-mediated material resorption. We report here the results of a prospective study aiming to evaluate the degree of fusion obtained by the use of a magnesium-doped hydroxyapatite product to achieve postero-lateral fusion in degenerative spine diseases. Second aim of the study was to evaluate of the safety profile of the bone graft in spinal arthrodesis procedures.*
Materials and methods. *A prospective, spontaneous, observational, post-marketing clinical study was conducted. Twenty subjects were included in the study and underwent surgery for postero-lateral fusion with the use of the magnesium-doped hydroxyapatite bone graft.*
Results. *At 12 months follow-up, more than 70% of fusion was reached. A statistically significant improvement was observed for clinical parameters related to the improvement of life quality following spinal fusion.*
Conclusions. *The magnesium-doped hydroxyapatite bone graft substitute SintLife represents a good alternative to autologous bone graft. The device can be safely used alone performing in a similar manner as the "gold standard" autologous bone.*

KEY WORDS: *spinal fusion, hydroxyapatite bone graft, magnesium-hydroxyapatite.*

□ INTRODUCTION

Lumbar spinal fusion (i.e. arthrodesis) is one of the most used surgical procedures for the treatment of deformities, trauma and degenerative diseases with instability of the spine[1,2]. A successful fusion (intended as radiographically detectable neo-formation of trabecular bone, in particular at the interface with the receiving bone surface) depends on the characteristics and properties of the bone graft used, as well as on the surgical technique employed. The biological process leading to new bone formation is

Corrispondence: Valentina Canella, Clinical Department, Fin-Ceramica Faenza S.p.A., via Ravegnana 186, SP 302, 48018 Faenza (RA), Italy, ph. +39-0546 607355, e-mail: valentina.canella@finceramica.it
Progress in Neurosciences 2021; 6 (1-4): 27-35. *ISSN: 2240-5127.*

LIST OF ACRONYMS AND ABBREVIATIONS: **β-TCP** = Beta-TriCalcium Phosphate; **BMPs** = Bone Morphogenetic Proteins; **CaP** = Calcium Phosphate; **CE-marked** = Conformitè Europëenne (European conformity)-marked; **CT** = Computed Tomography; **DBM** = Demineralized Bone Matrix; **HA** = HydroxyApatite; **hMSCs** = human Mesenchymal Stem Cells; **ICBG** = Iliac Crest Bone Graft; **Mg** = Magnesium; **ODI** = Oswestry Disability Index; **ROI** = Region of Interest; **VAS** = Visual Analog Scale.

characterized by three critical elements: the osteogenic potential, the presence of osteoinductive factors and the osteoconductive properties of the bone graft used, either if it is autologous bone from the own patient or a synthetic bone graft substitute.

The ideal bone substitute shows all three of these properties, associated with excellent compatibility and biological safety[3,4].

In this perspective, autologous bone graft from the iliac crest is considered the "gold standard" because of these properties[5-9]. Nevertheless, autologous bone harvesting has shown limitations and significant drawbacks, including superficial infection, wound complications, sensory abnormalities, persistent pain, hematomas, as well as need for reoperation, scarring, graft site fracture[8-11].

To overcome these limitations, different alternatives have been developed, clinically tested and currently available on the market, including allograft[4,12], bone morphogenetic protein, DBM[13-15], BMPs[16,17], hMSCs and bioceramics[14].

Although extensively studied, clinical data available for all these materials are often heterogeneous in quality, type of study, evaluations performed and conclusions reached[11,13,15,18].

Bioactive ceramics (i.e. tricalcium phosphate, calcium phosphate, calcium sulphate, hydroxyapatite and collagen)[19] are synthetic products which have been developed as osteoconductive scaffolds with chemico-physical properties very similar to the mineral component of human bone[18,20-22]. These biomaterials are able to stimulate cell proliferation and differentiation, bone tissue regeneration/remodelling while undergoing in the meantime slow resorption. Among all, HA is the most similar, for chemico-physical composition and stoichiometric formula (Ca/P ratio = 1.67), to the mineralized phase of human bone. To further improve their features, new generation HA-based biomaterials have been developed with superior properties, strongly influenced by the nature of components, the composition and the morphology. Calcium ions, phosphate ions, and hydroxyl groups can be replaced by other ions, and studies in animal models have demonstrated that HA-substituted ions enable the crystal cell structure of ceramic derivatives to become unstable and more biologically active, thereby promoting rapid cell-mediated material resorption, new bone formation and remodelling[23].

Magnesium is certainly one of the most important bivalent ion associated with biological apatite: it is one of the most abundant minerals in the human body and approximately 50% of Mg^{2+} is naturally present in the composition of bone tissue.

Mg^{2+} enables the HA crystal cell structure to become unstable and more biologically active, promoting rapid cell-mediated material resorption, new bone formation and remodelling by cross-talking with progenitor cells at the molecular level. In vitro experiments have revealed an active interaction between Mg-HA biomaterials and hMSCs, with an improvement of metabolic cell activity[24,25]; preclinical studies in large animal models[26,27] have demonstrated good osteo-integration and the deposition of new bone tissue.

In this context, an Mg-HA bone graft substitute has been employed to achieve fusion in a cohort of patients undergoing postero-lateral fusion for degenerative lumbar spine diseases.

Aim of the present prospective, observational study was to evaluate the performance (in terms of fusion and improvement of life quality) and safety of the Mg-HA bone graft substitute (*SintLife, Finceramica, Faenza, S.p.A., Italy*) in procedures of spinal stabilization.

□ MATERIALS AND METHODS

■ **Clinical study design.** A prospective, spontaneous, observational, post-marketing clinical study was conducted at our Centre from March 2020, after approval of the local Ethic Committee, to December 2021. Consecutive patients who had indications for single or multi-level posterolateral spinal fusion due to degenerative lumbar spine diseases were screened to be enrolled in the study. For all the patients included (according to inclusion/exclusion criteria), informed consent was required.

The enrolment period was from March 2020 to December 2021, and patients were followed up from the pre- pre-operative visit to 12 months postoperative, with intermediate post-operative visit just after surgery and at 6 months. Specific inclusion criteria were as follows: skeletally mature subjects, at least 18 years of age at the time of surgery, affected by symptomatic spinal diseases, due to degenerative or oncological conditions or trauma, and requiring interbody postero-lateral fusion at C2-T1 tract with stabilization systems and bone graft; patients for which autologous bone was not sufficient to allow arthrodesis and requiring the use of a bone graft substitute; patients participating in the study who provided informed signed consent. Patients' exclusion criteria were applied in case of local or systemic infections, inflammatory or autoimmune disease, hypercalcemia, coagulation/metabolic disorders, insulin-dependent diabetes, previously known allergy to calcium phosphate salts, drugs or magnesium, active neoplasia. The sample size population was estimated in 20 subjects to be included. However, due to the concomitant development of the COVID-19 pandemic in 2020 (which resulted in a slowdown of surgical activities in our Centre) a delay in patient enrollment has been observed. For this reason, in order to respect the timelines of the study, on September 2021 we decided to retrospectively enroll the remaining number of patients that were missing to reach the sample size population estimated. This was done by screening all the subjects that underwent postero-lateral fusion with the device under analysis from March 19, 2020 (day of study initiation) backwards, and responding to the inclusion and exclusion criteria settle in the study.

■ **Follow-up plan.** A schematic view of the visits planned during the study is reported in Table 1. According to the chemico-physical properties of the Mg-HA bone graft SintLife, which is reported by the Manufacturer to be resorbed in a period of about 6-18 months, follow-up was set up at 12 months.

■ **Surgical procedure.** Conventional posterior approach for lumbar spinal fusion was performed. After the positioning of pedicle screws, decompression of the cauda and nerve roots was achieved with a hemilaminectomy and foraminotomy. SintLife was put on the hemi-laminae and transvers process on the contralateral side of the hemilaminectomy. During surgery, SINTlife was mixed with antibiotics (rifampicin in powder, 60 mg) in order to reduce the any risk of early infection.

■ **Biomaterial.** SintLife is an implantable, non-active, CE-marked medical device that acts as a bone graft substitute. The device is made of biomimetic HA enriched with magnesium ions (Mg^{2+}) in a similar amount as in human bones. The device interacts with bone tissue-forming cells to promote bone regeneration. Its biomimetic chemical composition, structured geometry, and surface properties allow new bone formation: the presence of Mg^{2+} ions, introduced into the HA crystalline cell in the same position and percentage found in the mineral phase of human bone, makes the device unstable and biologically active, thus favoring interaction with key proteins involved in osteogenesis and bone remodeling. The device is therefore quickly resorbed by osteoprogenitor cells in a physiologically adequate period of time (6-18 months), while sustaining new bone formation by osteoprogenitor cells.

■ **Radiographic and clinical outcomes measure.** Radiographs were taken pre-operatively, immediately following surgery and at 1-year follow-up, and were reviewed by an independent radiologist. Fusion was defined as a continuous trabecular bone bridge in the entire fusion area with no radiolucency between the graft bone and the vertebral bone through diagnostic imaging (TC scan). We used the classification of Brantigan and Steffee[28], as defined in Table 2.

Study visits flow chart	Pre-op.	Immediate post-op. surgery	6 months FU	12 months FU
Assessment of patients eligibility (Inclusion/exclusion criteria)	X			
Informed consent signature	X			
Patients anamnesis	X			
Surgical report		X		
ODI and VAS scores recording	X		X	X
CT acquisition	X	X		X
Adverse events recording		X	X	X

Table 1. Flow chart visits. *Legend:* CT = Computed Tomography; FU = follow-up; ODI = Oswestry Disability Index; post-op. = post-operative; pre-op. = pre-operative; VAS = Visual Analog Scale.

Classification	Description
A. Obvious radiographic pseudoarthrosis	Pseudoarthrosis, collapse of construct, loss of disc height, vertebral slip, broken screw, displacement of the cage, resorption of bone graft
B. Probable pseudoarthrosis	Significant resorption of bone graft, major lucency or gap visible in the fusion area > 2 mm
C. Radiographic status uncertain	A small lucency or gap may be visible with at least half of the graft area showing no lucency between the graft bone and the vertebral bone
D. Probable radiographic fusion	Bone bridges the entire fusion area with at least the density originally achieved at surgery. There should be no lucency between the graft bone and the vertebral bone
E. Radiographic fusion	The bone in the fusion area is more dense and more mature than originally achieved at surgery; there is no interface between the donor bone and the vertebral bone: a sclerotic line between the graft bone and the vertebral bone indicates solid fusion. Other indicators of solid fusion is fusion at facet joints and anterior progression of the graft in the disc

Table 2. Brantigan and Steefe classification of spinal fusion.

The following clinical parameters were evaluated: the VAS for patient's pain intensity and the ODI for the quantification of patient's disability for low back pain, which were assessed pre-operatively, at 6 and 12 months post-operative.

■ **Safety assessment.** The safety of SintLife was assessed by the rate of adverse events and complications recorded during the whole study period.

■ **Statistical analysis.** The sample size was defined according to the real clinical practice of the clinical site developing the study, and estimated in 20 subjects to be included and enrolled. Considering the heterogeneity of the patient population included in the study, no specific statistical analyses have been carried out. Descriptive statistical analysis has been provided for clinical scores (VAS, ODI).

Results are presented as number, mean ± standard deviation and percentage, as appropriate. Changes from baseline to follow-up scores were analysed using the t-student test. The level of statistical significance was set at $p < 0.05$. The SAS software 9.201 was used. Assessment of fusion and the incidence and type of any adverse event recorded has been reported.

□ RESULTS

The study was approved by the local Ethic Committee and informed signed consent was obtained from each patient. Twenty patients were included in the study, of which 13 male (75%) and seven female (35%. Mean age at the time of surgery was 64.7-year-old (range 36-85). Eighty-five percent of subjects were treated for lumbar stenosis, 10% for pathologies following trauma and 5% for infective diseases. Forty-five percent of subjects underwent one level of fusion, 40% had 2 levels and 15% four or more fusion levels. The bone graft substitute SintLife was used alone to allow fusion. The followings were identified as possible risk factors: hypertension (recorded in 30% of cases), diabetes (25% of cases), previous known allergies (5%). Out of the total number of 20, 10% of patients were contemporarily affected by more than one risk factor. The full-set population analysed was of 20 subjects. The full-set of "completers" (i.e. subjects completing all the post-operative visits) was of 15 patients.

Bony fusion was evaluated by an independent radiologist on TC images at 12 months follow-up, for the full-set of "completers" (N = 15). A successful fusion was considered where Brantingan score of 5, 4 and 3 was reached. Accordingly, 11 subjects (73.4%) reached a successful fusion (Table 3). The results were confirmed by quantification of the structural homogeneity of the bone graft area (ROI) (data not shown). The VAS score at baseline in the full-set group was 7.2 ± 1.3, 2.1 ± 1.15 at 6 months follow-up and 0.5 ± 0.5 at 12 months follow-up, with statistically significant differences between pre-baseline and 6 months ($p < 0.01$) and between baseline and 12 moths post-operative ($p = 0.001$) (Table 4 and Figure 1).

The ODI score in the full-set group was 25.1 ± 6.6 at baseline, 8 ± 4.55 at 6 months follow-up and 1.9 ± 1.8 at 12 months follow-up, with statistically significant

Brantingan score	Number of patients reaching fusion according to the Brantingan score	%
5	2	13.3
4	6	40
3	3	20
2	4	26.7
1	0	0

Table 3. Number of patients reaching fusion.

differences between pre-baseline and 6 months ($p < 0.001$) and between baseline and 12 moths post-operative ($p < 0.001$) (Table 5 and Figure 2).
The same analysis was performed for the full-set "completers", which is of 15 subjects. ROI values for ODI and VAS were equivalent to the full-set population. At 6 and 12 months follow-up, the trend was confirmed for both VAS and ODI scores.
Clinical scores (ODI, VAS) were calculated for subgroup populations (i.e. < 65 years old versus ≥ 65 year old) No statistically significant differences were recorded (data not shown).

■ **Safety Analysis.** One minor adverse event was recorded (i.e. superficial inflammation). The event was evaluated by the Surgeon as partially related SintLife. The event was treated with anti-inflammatory therapy and no surgical revision was required.

☐ DISCUSSION

There are varieties of bone graft substitutes that are available for use in spine fusion surgery. In general, these types of bone graft are a synthetic and, recently, most of them are naturally-occurring products. During years, the interest in developing and refining bone graft substitute options for use in lumbar spinal fusion surgery procedures has improved, aimed at eliminating the need to harvest the patient's own bone, thus potentially reducing the risk and pain associated with the procedure and resulting in higher fusion rates.
Among the synthetic options, the family of ceramic biomaterials represents and interesting alternative, due to their chemico-physical features. Ceramic-based bone graft substitutes (CaP, β-TCP, HA) can vary according to the different compositions, manu-

Visual analogue scale	N.	Mean	SD	Median	Min.	Max.	P
Pre-operative	20	7.2	1.27	7.5	5	9	
6 months	20	2.1	1.15	2	0	4	< 0.001
12 months	15	0.5	0.52	1	0	1	0.001

Table 4. Visual analog scale. *Legend:* Max = Maximum; Min = Minimum; N. = Number; SD = Standard Deviation.

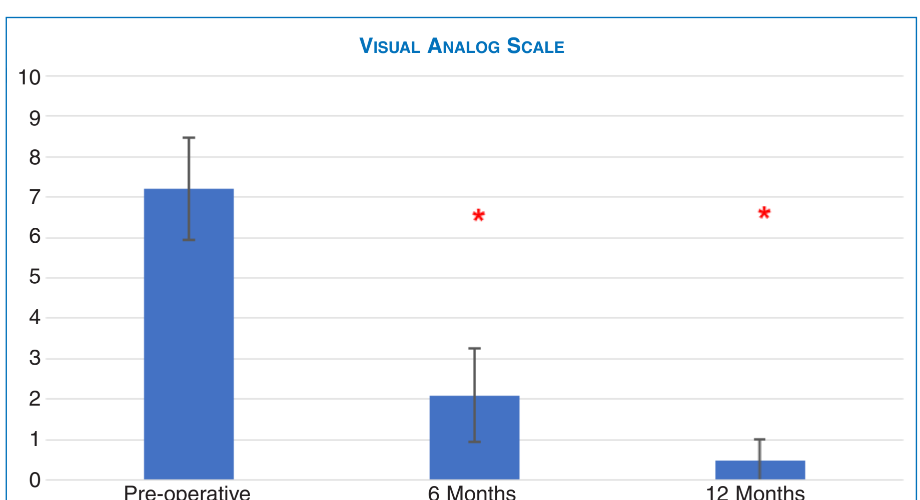


Figure 1. Plot of Visual Analog Scale scores evaluated pre-operatively, at 6 and 12-months follow-up. The *red asterix* highlights a significant difference between post-operative and pre-operative values.

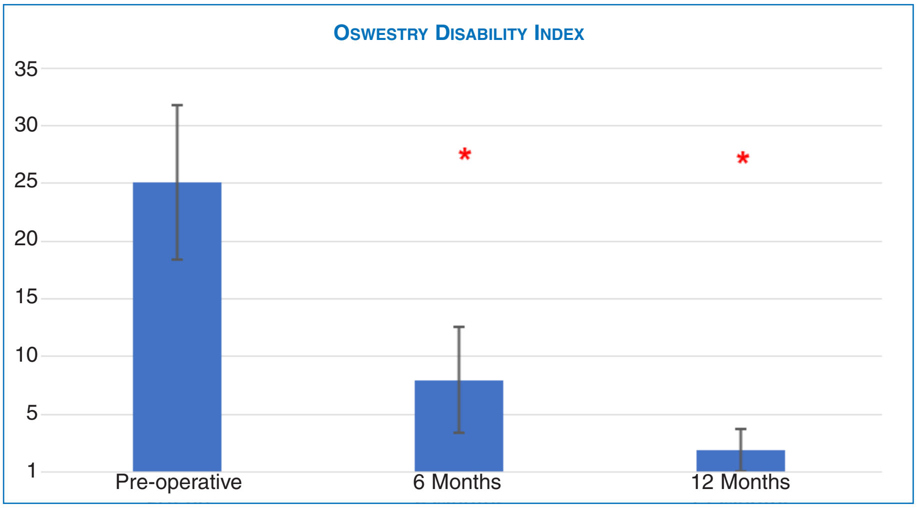


Figure 2. Oswestry Disability Index scores evaluated pre-operatively, at 6 and 12 months follow-up. The *red asterix* highlights a significant difference between post-operative and pre-operative values.

facturing, porosity and structure, but they all mainly have a composition which mimics the mineral phase of bone. Ceramics are osteoconductive and osteo-integrative, but not osteogenic nor osteoinductive. They can have pores with different sizes, which are critical for osteoprogenitor cells migration and differentiation in functional osteoblasts.

Hydroxyapatite, a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, has a stoichiometric formula (Ca/P ratio = 1.67) and a chemico-physical composition which is very similar to the mineral phase of human bone. The porous structure of HA, with its macropore network and the micropores interconnection, induces rapid vascular and mesenchymal invasion, providing a specific cell flow and the optimal environment for cells to attach, proliferate, and finally differentiate into functional osteoblasts. HA shows high biomimetic properties, osteoconductive potential and excellent biocompatibility, is biodegradable and pose virtually no risk of infection or donor site morbidity[(12)].

In the last decades, research into synthetic material composites as bone graft substitutes has increased due to the ability to manipulate composite properties, and "new generation" biomaterials have been developed, to the aim of "mimicking" the osteoregenerative processes typically found in the human bone mineral turn-over. Magnesium is certainly one of the most important bivalent ions associated with biological apatite, being one of the most abundant minerals in human bones. Mg^{2+} enables the HA crystal cell structure to become unstable and more biologically active, promoting rapid cell-mediated material resorption, new bone formation and remodelling by cross-talking with progenitor cells at the molecular level[(29,30)].

For this reason, in our Institute we have approached to a HA-based bone graft substitute enriched in Magnesiun named SintLife. The device has been used safely in a number of clinical cases and has become a commonly used bone graft substitute in procedures of spinal arthrodesis.

Previous pre-clinical experiments tested the performance and safety of the device. In vitro tests revealed an active interaction between SintLife and hMSCs, with an improvement of cell metabolic activities and bone remodelling[(24,25)]. An *in vivo* study on sheep treated for posterolateral fusion with SintLife or autologous bone showed the deposition of new bone tissue provided by SintLife, without quali-

Oswestry disability index	N.	Mean	SD	Median	Min.	Max.	P
Pre-operative	20	25.1	6.66	25	15	37	
6 months	20	8.0	4.55	7	0	17	< 0.001
12 months	15	1.9	1.79	3	0	4	< 0.001

Table 5. Oswestry disability index. *Legend:* N. = Number; Max = Maximum; Min = Minimum; SD = Standard Deviation.

tative and quantitative differences with respect to new bone formed with autologous bone graft[31]. Other preclinical studies[26,27] demonstrated good osteointegration and deposition of new bone tissue by the use of SintLife.

In the present observational, prospective clinical study, we investigated the use of Sintlife used alone in procedures of postero-lateral fusion, showing 73.4% of successful fusion reached, which is in line with previous findings , reporting a fusion rate of 70-90% obtained by the use of hydroxyapatite-based bone grafts used alone, as compared to autograft[32]. Although only few studies to date have evaluated successful fusion of HA-based bone grafts used alone, data from this prospective study confirm the efficacy of the Mg-HA bone graft SintLife in arthrodesis procedures. The success of fusion was also confirmed by an improvement of those values (i.e. ODI and VAS) which are used in the clinical practice to measure patients' disability, pain and quality of life before and after spinal stabilization by arthrodesis. No statistically significant differences were reported when considering the age (adults versus elderly patients) at the time of surgery, highlighting a satisfactory reaction to the surgical treatment.

The safe profile of SintLife is confirmed by the lack of adverse events related to the product, as also previously demonstrated in a Post-Marketing Surveillance analysis[33] involving the device and confirmed in the present study. Only one minor event (i.e. a local infection treated with antibiotic therapy) has been recorded during the study, possibly related to the patient's clinical conditions, which may affect the post-operative course. Available literature reports about cases of inflammatory reactions following bone grafts substitutes use for spinal fusion[34-36]. These events are always related to previous patients' clinical inflammatory conditions.

Nonetheless, hypersensitivity to some components of the device have always to be taken into account, and are usually reported in the Manufacturer Instruction For Use.

Limitations of the study are mainly related to the low number of subjects enrolled and then completing all the follow-up visits: possible causes can be found in the concomitant worldwide pandemic COVID-19, which drastically reduced clinical activities during the period of the study conduction. By the other side, patients satisfied by the improvement of their quality of life following spinal fusion tend to avoid subsequent visits.

Being understood that future searches are needed to further investigate the use of SintLife in procedures of spinal fusion on larger populations, the biomaterial has shown good performance in terms of fusion achievement and improvement of clinical scores. The satisfying biocompatibility as well as the safe profile confirm the use of the device in spinal fusion procedures.

□ CONCLUSIONS

In conclusion, the Mg-doped HA bone graft substitue SintLife represents a good alternative to autologous bone graft. The device can be safely used alone performing in a similar manner as the "gold standard" autologous bone.

□ REFERENCES

1. Rajaee SS, Bae HW, Kanim LE, Delamarter RB. Spinal fusion in the United States: analysis of trends from 1998 to 2008. Spine 2012; 37 (1): 67-76.
2. Yoshihara H, Yoneoka D. National trends in the surgical treatment for lumbar degenerative disc disease: United States, 2000 to 2009. Spine J 2015; 15 (2): 265-271.
3. Ludwig SC, Kowalski JM, Boden SD. Osteoinductive bone graft substitutes. Eur Spine J 2000; 9 (Suppl 1): S119-125.
4. Park JJ, Hershman SH, Kim YH. Updates in the use of bone grafts in the lumbar spine. Bull Hosp Jt Dis 2013; 71 (1): 39-48.
5. Ahlmann E, Patzakis M, Roidis N, Shepherd L, Holtom P. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J Bone Joint Surg Am 2002; 84 (5): 716-720.
6. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Complications of iliac crest bone graft harvesting. Clin Orthop Relat Res 1996; 329: 300-309.
7. Banwart JC, Asher MA, Hassanein RS. Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. Spine 1995; 20 (9): 1055-1060.
8. Dimar JR 2nd, Glassman SD, Burkus JK, Pryor PW, Hardacker JW, Carreon LY. Two-year fusion and clinical outcomes in 224 patients treated with a single-level instrumented posterolateral fusion with iliac crest bone graft. Spine J 2009; 9 (11): 880-885.
9. Kim DH, Rhim R, Li L, Martha J, Swaim BH, Banco RJ, Jenis LG, Tromanhauser SG. Prospective study of iliac crest bone graft harvest site pain and morbidity. Spine J 2009; 9 (11): 886-892.
10. Gruskay JA, Basques BA, Bohl DD, Webb ML, Grauer

JN. Short-term adverse events, length of stay, and readmission after iliac crest bone graft for spinal fusion. Spine 2014; 39 (20): 1718-1724.

11. Miyazaki M, Tsumura H, Wang JC, Alanay A. An update on bone substitutes for spinal fusion. Eur Spine J 2009; 18 (6): 783-799.
12. Gupta A, Kukkar N, Sharif K, Main BJ, Albers CE, El-Amin Iii SF. Bone graft substitutes for spine fusion: a brief review. World J Orthop 2015; 6 (6): 449-456.
13. Abdullah KG, Steinmetz MP, Benzel EC, Mroz TE. The state of lumbar fusion extenders. Spine 2011; 36 (20): E1328-1334.
14. Fischer CR, Cassilly R, Cantor W, Edusei E, Hammouri Q, Errico T. A systematic review of comparative studies on bone graft alternatives for common spine fusion procedures. Eur Spine J 2013; 22 (6): 1423-1435.
15. Hsu WK, Nickoli MS, Wang JC, Lieberman JR, An HS, Yoon ST, Youssef JA, Brodke DS, McCullough CM. Improving the clinical evidence of bone graft substitute technology in lumbar spine surgery. Global Spine J 2012; 2 (4): 239-248.
16. Carragee EJ, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. Spine J 2011; 11 (6): 471-491.
17. Kannan A, Dodwad SN, Hsu WK. Biologics in spine arthrodesis. J Spinal Disord Tech 2015; 28 (5): 163-170.
18. Alsaleh KA, Tougas CA, Roffey DM, Wai EK. Osteoconductive bone graft extenders in posterolateral thoracolumbar spinal fusion: a systematic review. Spine 2012; 37 (16): E993-1000.
19. Nickoli MS, Hsu WK. Ceramic-based bone grafts as a bone grafts extender for lumbar spine arthrodesis: a systematic review. Global Spine J 2014; 4 (3): 211-216.
20. Gao C, Deng Y, Feng P, Mao Z, Li P, Yang B, Deng J, Cao Y, Shuai C, Peng S. Current progress in bioactive ceramic scaffolds for bone repair and regeneration. Int J Mol Sci 2014; 15 (3): 4714-4732.
21. Kaiser MG, Groff MW, Watters WC 3rd, Ghogawala Z, Mummaneni PV, Dailey AT, Choudhri TF, Eck JC, Sharan A, Wang JC, Dhall SS, Resnick DK. Guideline update for the performance of fusion procedures for degenerative disease of the lumbar spine. Part 16: bone graft extenders and substitutes as an adjunct for lumbar fusion. J Neurosurg Spine 2014; 21 (1): 106-132.
22. Korovessis P, Koureas G, Zacharatos S, Papazisis Z, Lambiris E. Correlative radiological, self-assessment and clinical analysis of evolution in instrumented dorsal and lateral fusion for degenerative lumbar spine disease. Autograft versus coralline hydroxyapatite. Eur Spine J 2005; 14 (7): 630-638.
23. Nandi SK, Roy S, Mukherjee P, Kundu B, De DK, Basu D. Orthopaedic applications of bone graft and graft substitutes: a review. Indian J Med Res 2010; 132: 15-30.
24. Barbanti Brodano G, Mazzoni E, Tognon M, Griffoni C, Manfrini M. Human mesenchymal stem cells and biomaterials interaction: a promising synergy to improve spine fusion. Eur Spine J 2012; 21 (Suppl. 1): S3-9.
25. Manfrini M, Di Bona C, Canella A, Lucarelli E, Pellati A, D'Agostino A, Barbanti-Bròdano G, Tognon M. Mesenchymal stem cells from patients to assay bone graft substitutes. J Cell Physiol 2013; 228 (6): 1229-1237.
26. Sartori M, Giavaresi G, Tschon M, Martini L, Dolcini L, Fiorini M, Pressato D, Fini M. Long-term in vivo experimental investigations on magnesium doped hydroxyapatite bone substitutes. J Mater Sci Mater Med 2014; 25 (6): 1495-1504.
27. Pola E, Nasto LA, Tampieri A, Lattanzi W, Di Giacomo G, Colangelo D, Ciriello V, Pagano E, Spinelli S, Robbins PD, Logroscino G. Bioplasty for vertebral fractures: preliminary results of a pre-clinical study on goats using autologous modified skin fibroblasts. Int J Immunopathol Pharmacol 2011; 24 (1 Suppl. 2): 139-142.
28. Brantigan JW, Steffee AD. A carbon fiber implant to aid interbody lumbar fusion. Two-year clinical results in the first 26 patients. Spine 1993; 18 (14): 2106-2107.
29. Kurien T, Pearson RG, Scammell BE. Bone graft substitutes currently available in orthopaedic practice: the evidence for their use. Bone Joint J 2013; 95-B (5): 583-597.
30. Landi E, Logroscino G, Proietti L, Tampieri A, Sandri M, Sprio S. Biomimetic Mg-substituted hydroxyapatite: from synthesis to in vivo behaviour. J Mater Sci Mater Med 2008; 19 (1): 239-247.
31. Bròdano GB, Giavaresi G, Lolli F, Salamanna F, Parrilli A, Martini L, Griffoni C, Greggi T, Arcangeli E, Pressato D, Boriani S, Fini M. Hydroxyapatite-based biomaterials versus autologous bone graft in spinal fusion: an in vivo animal study. Spine 2014; 39 (11): E661-E668.
32. Buser Z, Brodke DS, Youssef JA, Meisel HJ, Myhre SL, Hashimoto R, Park JB, Tim Yoon S, Wang JC. Synthetic bone graft versus autograft or allograft for spinal fusion: a systematic review. J Neurosurg Spine 2016; 25 (4): 509-516.
33. Barbanti Brodano G, Griffoni C, Zanotti B, Gasbarrini A, Bandiera S, Ghermandi R, Boriani S. A post-market surveillance analysis of the safety of hydroxyapatite-derived products as bone graft extenders or substitutes for spine fusion. Eur Rev Med Pharmacol Sci 2015; 19 (19): 3548-3555.
34. Feng JT, Yang XG, Wang F, He X, Hu YC. Efficacy and safety of bone substitutes in lumbar spinal fusion: a systematic review and network meta-analysis of randomized controlled trials. Eur Spine J 2020; 29 (6): 1261-1276.
35. Mokawem M, Katzouraki G, Harman CL, Lee R. Lumbar interbody fusion rates with 3D-printed lamellar titanium cages using a silicate-substituted calcium phosphate bone graft. J Clin Neurosci 2019; 68: 134-139.
36. Bolger C, Jones D, Czop S. Evaluation of an increased strut porosity silicate-substituted calcium phosphate,

SiCaP EP, as a synthetic bone graft substitute in spinal fusion surgery: a prospective, open-label study. Eur Spine J 2019; 28 (7): 1733-1742.

Conflict of Interest Statement: *The Authors declare they have no financial or other conflict of interests in relation to this research and its publication.*

Original article

□ Anterior cervical fusion using magnesium-enriched hydroxyapatite: a two-year follow-up in 75 cases

V. CIOFFI*, A. BOCCHETTI*, A. NATALONI**, V. CANELLA**, M. SANDRI***, R. DE FALCO*

* *"S. Maria delle Grazie" Hospital, ASL Napoli 2 Nord, Pozzuoli (Naples), Italy*
** *Finceramica Faenza S.p.A., Faenza (Ravenna), Italy*
*** *National Research Council (Centro Nazionale delle Ricerche: CNR) - Institute of Science and Technology for Ceramics (ISTEC), Faenza (Ravenna), Italy*

SUMMARY: Aims. *Anterior cervical discectomy and fusion is a surgery indicated to remove a herniated or degenerative disc in the neck. Numerous graft extenders are available on the market as alternatives to the patient's own autograft bone. Among all, calcium phosphate salts such as hydroxyapatite and β-tricalcium phosphate can be inserted in cages and allow vertebral fusion. Aim of the present study was to evaluate the safety and efficacy of a new generation biomimetic hydroxyapatite enriched with magnesium ions inserted in cages for spinal fusion.*

Materials and Methods. *A series of consecutive patients undergoing anterior cervical discectomy was selected to be treated with the use of an magnesium-doped ydroxyapatite bone graft material inserted in cage. Clinical and radiologic data were collected immediately after surgery, then up to 2 year after surgery. The primary endpoint (i.e. safety) was considered as lack of any adverse event leading to revision surgery or re-hospitalization during the follow-up period. The secondary endpoint (i.e. performance or evidence of fusion) was considered as lack of cage mobilizations recorded during the follow-up period.*

Results. *Seventy-five patients underwent interbody spinal fusion for the treatment of anterior cervical discectomy. A minimum 2-year follow-up was recorded for all the patients. Radiographic images showed the stability of the implant, as result of successful surgery. No adverse events (cage mobilization, infection, etc.) were recorded.*

Conclusions. *Mg-hydroxyapatite bioceramics show osteoconductive properties and a safety profile when employed in anterior cervical discectomy procedures.*

KEY WORDS: *Anterior cervical discectomy and fusion, Fusion, Magnesium-enriched hydroxyapatite.*

□ INTRODUCTION

Spondylosis is the general degeneration of the spine that can occur in joints, discs, and bones of the spine as we age. Conservative management, such as anti-inflammatories or physical therapy, is the preferred and often only required intervention. In unresponsive patients, surgery is indicated[8]. Anterior cervical discectomy and fusion is a surgery indicated to remove a herniated or degenerative disc in the neck.

Corrispondence: Dr.ssa Valentina Canella, Fin-Ceramica Faenza SpA, via Ravegnana 186, 48018 Faenza (RA), Italia, phone: +39-(0)546-645411, fax +39-(0)546-46355, e-mail: valentina.canella@finceramica.it
Progress in Neurosciences 2021; 6 (1-4): 37-42. *ISSN: 2240-5127.*

LIST OF ACRONYMS AND ABBREVIATIONS: **ACDF** = Anterior Cervical Discectomy and Fusion; **ANOVA** = ANalysis Of Variance; **BMI** = Body Mass Index; **CaP** = Calcium Phosphate; **HA** = HydroxyApatite; **Mg** = Magnesium.

This surgical procedure allows removing the damaged disc from the front (anterior) of the spine through the throat area. After the disc is removed, the space between the bony vertebrae is empty and a spacer bone graft is inserted to fill the open disc space and prevent the vertebrae from collapsing. The graft serves as a bridge between the two vertebrae to create a spinal fusion. The system "bone graft-vertebrae" are fixed in place with metal plates and screws. The instrumentation and fusion work together and, after 3 to 6 months, the bone graft should join the two vertebrae and form one solid piece of bone.

Bone grafts available to be used in ACDF can come from different sources. Typically, autologous bone is considered the "gold standard" choice, because of the osteogenic, osteoinductive and osteoconductive properties, which allow new bone formation[8,9,13,14], few incidences of graft complication and biocompatibility, no risk of disease transmission or immunogenic reactions[12]. However, drawbacks such as risk of harvest site morbidity, infection, hematoma, pelvic fracture, limitations with supply and others, can limit its use[14].

Among the possible graft alternatives available, synthetic bone grafts such as calcium phosphate derivatives and ceramics have been widely employed in fusion procedures, because of their biocompatibility, no risk of infection, the unlimited supply and, most important, the lack of donor site morbidity[7,13].

Based upon the natural occurring calcium salts and hydroxyapatite found in human bone, ceramics have been widely investigated for use in the cervical spine as well, showing good results in terms of fusion performance and clinical outcomes[6,15,29].

In the last decades, new generations HA-based biomaterials have been developed with superior properties, strongly influenced by the nature of components, the composition and the morphology. Calcium ions, phosphate ions, and hydroxyl groups can be replaced by other ions, and studies in animal models have demonstrated that HA-substituted ions enable the crystal cell structure of ceramic derivatives to become unstable and more biologically active, thereby promoting rapid cell-mediated material resorption, new bone formation and remodeling[23].

Magnesium is certainly one of the most important bivalent ions associated with biological apatite: it is one of the most abundant minerals in the human body and approximately 50% of Mg^{2+} is naturally present in the composition of bone tissue. Mg^{2+} enables the HA crystal cell structure to become unstable and more biologically active, promoting rapid cell-mediated material resorption, new bone formation and remodeling by cross-talking with progenitor cells at the molecular level[10,16,17]. Conversely, Mg^{2+} depletion affects all stages of skeletal metabolism, causing cessation of bone growth, decrease in osteoblastic activities, osteopenia and bone fragility. For all these reasons, Mg-HA has become of great interest in the development of effective bone substitutes[10,17]. In vitro experiments revealed an active interaction between Mg-HA biomaterials and human mesenchymal stem cells with an increase of cell metabolic activity[3,18], whereas preclinical studies in large animal models[5,24,27] demonstrated good osteointegration and the deposition of new bone tissue in spinal procedures.

In this panorama, an Mg-doped HA-based bone graft material (*SintLife, FinCeramica Faenza, S.p.A.*) has been employed in our institute for interbody spinal fusion procedures. In vivo animal models to assess spinal fusion with the device have shown that Mg-HA led to the deposition of new bone tissue without qualitative and quantitative differences as compared to newly bone formation with the use of autograft bone[5].

Due to the conspicuous casuistry of patients treated at our Institution with the above-mentioned biomaterial, the present retrospective case series aims to demonstrate the safety and efficacy at 2 years follow-up of the Mg-HA doped SintLife as bone graft substitute inserted in cages for cervical interbody fusion.

☐ AIMS

Aim of the present study was to evaluate the safety and efficacy of a new generation biomimetic hydroxyapatite enriched with magnesium ions inserted in cages for spinal fusion.

☐ MATERIALS AND METHODS

■ **Patient population.** This is a retrospective case series of patients undergoing ACDF with the use of an Mg-doped HA bone graft material inserted in cage at the Department of Neurosurgery, “Santa Maria delle Grazie” Hospital in Pozzuoli, Naples, Italy, between 2017 and 2019. Data were gathered through the review of patients’ case notes and relevant data records. Patients were evaluated preoperatively: baseline characteristics (age, gender, BMI), smoking habits, and previous surgical procedures undertaken) were recorded before surgery. No inclusion or exclusion criteria were selected.

■ **The device description.** SINTlife is a Class III, new generation biomimetic hydroxyapatite enriched with magnesium (Mg^{2+}) ions and with a chemical and physical composition equivalent to the mineral constituent of human bones. Thanks to the specific biomimetic chemical composition and the micro-structural properties, SINTlife promotes new bone formation, high osteointegration kinetics and resorbable properties. The device, in form of putty, can be easily handled and stabilized in bone cavity sites. SintLife remains in situ for the time required for bone growth and maturation and, while sustaining osteo-progenitor cells remodeling, it resorbs over a physiologically appropriate period (6 to 18 months). As such, fast osteointegration kinetics of magnesium-enriched hydroxyapatite lead to a faster bone formation.

■ **Surgical procedure.** All surgical procedures were performed by the same senior surgeon using open anterior approach to the cervical spine. Patients were preoperatively treated with intravenous antibiotic treatment. All patients underwent decompression and spinal stabilization with the use of instrumented fixation supports (pedicle screws/rods) in addition to bone graft material (*SintLife, Fin-Ceramica Faenza S.p.A., Faenza, Italy*).

SintLife was placed in titanium cage prior to its placement. The cage was then placed in the interbody space to allow bony fusion. In all the cases, the cage was compressed, in order to reproduce the normal inward lordotic curvature of the spine column. The wound was sutured in three layers over two suction drainage tubes. The patients were intravenously treated with prophylactic antibiotic therapy immediately after surgery and mobilized 2 to 3 days after surgery.

■ **Results assessment**. Clinical and radiologic data were collected immediately after surgery, then up to 2 year after surgery. The primary endpoint (i.e. safety) was considered as lack of any adverse event leading to revision surgery or re-hospitalization during the follow-up period. The secondary endpoint (i.e. performance or evidence of fusion) was considered as lack of cage mobilizations recorded during the follow-up period. The secondary endpoint (i.e. evidence of fusion) was considered achieved if, during the follow up period, no mobilization of cages was reported, which is possible only in case of bone graft performing fusion.

■ **Statistical analysis.** Values are presented as mean values, minimum and maximum ranges, ratios or percentages, as appropriate. Analysis was performed with the use of the Friedman ANOVA test for comparisons among the two treatment groups. The level of statistical significance was set at $P < 0.05$. Data were analyzed with the use of Statistica 6 software (*StatSoft Inc, Tulsa, Oklahoma, USA*).

□ RESULTS

Between 2017 and 2019, 75 patients underwent interbody spinal fusion for the treatment of degenerative disc diseases at the Department of Neurosurgery, the Santa Maria delle Grazie Hospital in Pozzuoli, Naples, Italy. A minimum 2-year follow-up was recorded for all the patients. There were 45 male and 30 female (ratio 1,5:1), mean age 54 years (age range 28-79). Main aetiology was degenerative (74/75 cases, 98.7%) and only was case was treated because of traumatic causes. None of the subjects had smoking habits. Patients were operated for ACDF, all the patients received a Mg-HA based graft material inserted in cage. The majority of patients (40, 53%) underwent 1 level of fusion, 26 underwent 2 levels of fusion, 9 patients had 3 fusion levels.

At follow-up period, radiographic images showed the stability of the implant, as result of successful surgery and fusion processes taking place. No adverse events (cage mobilization, infection, etc.) were recorded.

□ DISCUSSION

The purpose of a spinal fusion (arthrodesis) is to link or weld bones together. Many spinal conditions cause instability and/or pain (e.g., degenerative disc disease, scoliosis, trauma, infection and neoplasia) and require treatment with a spinal fusion[21]. Among the different possible approaches, interbody fusion is an established treatment aimed at removing a disc that is the source of back or leg pain, and fuse spinal vertebrae with the addition of bone grafts. Anterior cervical discectomy and fusion is a type of neck surgery that involves removing a damaged disc to relieve spinal cord or nerve root pressure and alleviate corresponding pain, weakness, numbness, and tingling. A fusion surgery is performed at the same time as the discectomy operation in order to stabilize the cervical segment. The fusion procedure involves placing bone graft and/or implants where the disc originally was in order to provide stability and strength to the area.

Bone grafts can be divided into three main categories (autologous bone, allograft bone, synthetic bone grafts) based on where they are obtained (the patient's own bone, bone obtained from cadavers and sterilized for use, synthetic products that either assist or replace the need for autograft or allograft bone in a spine fusion, respectively). The choice of which type of bone graft to use is largely dependent upon where the fusion is done in the spine (in the cervical, thoracic, or lumbar spine), the surgical approach to the fusion (anterior or posterior) and the availability materials. Autograft bone (usually harvested from the iliac crest) is considered the "gold standard" material to provide fusion. In ACDF, a mean arthrodesis rate of 77% is reported[31]. In one-level non-instrumented procedures, autograft fusion rates are a reported 83-99%[26], but decreases with number of levels fused. Autologous bone shows relatively few incidences of graft complication, such as graft collapse or migration, and it is biocompatible, posing no risk of disease transmission or immunogenic reactions. Nonetheless, drawbacks related to the harvesting procedures are reported. In a retrospective study of one-level ACDF, Silber and colleagues found 26.1% of patients suffering from persistent pain and 15.7% experienced numbness at the harvest site[28]. Functional assessment revealed impairment in ambulation (12.7%) and other daily activities. A 30% of donor site pain is also reported[25], which caused patients to have longer hospital stay. Finally, the limited supply can affect its use, on behalf of synthetic materials, which unlimited quantity and evolution during decades (to provide safety profiles) has allowed their ordinary use in common clinical practice of spinal procedures. Among the synthetic bone grafts (also known as "bone graft substitutes"), ceramic derivatives like calcium phosphate and hydroxyapatite have been employed in ACDF. Kim et al.[15] reported good clinical results and solid fusion at 1 year follow up in a patient population of 70 subjects operated for ACDF with a 30% porous HA graft. No graft collapse was reported, encasement of the implant and formation of union were observed. Bruneau and colleagues[6] showed complete fusion occurred in 98% of one-level and 100% of two-level procedures in a clinical study employing HA in ACDF. Thalgott et al. reviewed 26 patients who received coralline HA with rigid plating for ACF[30], showing no graft complications and 100% of grafts incorporation at 36 months follow-up. Coralline HA inserted in carbon fiber cages showed complete fusion at 12 months with no complications, in one- or two-level procedures[1]. A prospective randomized trial by McConnell et al., which compared coralline HA with autograft, showed significant clinical improvement and similar fusion rates (HA 78%, autograft 79%) at 24 months follow-up[20].

In the present study, we show the performance of a Mg-doped HA (named SintLife) to achieve bony fusion, as manifested by radiographic images. Previous in vitro experiments demonstrate chemotactic activities[3,18], new bone formation and good osteointegrative properties in spinal procedures[5,24,27], supporting its use in clinical practice.

In the present study we also show the safety profile of SintLife employed in ACDF procedures, confirmed by the lack of adverse events (i.e. inflammatory reactions or infections, cages mobilization, patients' morbidity) recorded in the follow-up period (2 year), as previously reported in literature by others[2].

According to current literature[11,19], bone graft substitutes with strong bioactive features may give rise to inflammatory reactions. Mokawem et al.[22] reported a 9.7% complication rate with the use of a Si-doped CaP bone graft packed in spinal fusion procedures, with some cases requiring revision surgery. Worst results were provided by Bolger[4], which reported a 30% complication rate with the use of Si-CaP in PLF procedures. 7% of the events were related to the device and required revision surgery, suggesting that some ions-doped bioceramics shall give rise to complications, as compared to CaP-based materials without ions augmentation.

In the present work, we show no inflammatory reactions following the use of Mg-HA in ACDF, highlighting the safety profile of the device when used in conjunction with cages.

□ CONCLUSIONS

SintLife, an hydroxyapatite bioceramic enriched in magnesium, has shown osteoconductive properties and a safety profile when employed in ACDF procedures.

REFERENCES

1. Agrillo U, Mastronardi L, Puzzilli F. Anterior cervical fusion with carbon fiber cage containing coralline hydroxyapatite: preliminary observations in 45 consecutive cases of soft-disc herniation. J Neurosurg 2002; 96 (3 Suppl.): 273-276. doi: 10.3171/spi.2002.96.3.0273
2. Barbanti Brodano g., Griffoni C, Zanotti B, et al. A post-market surveillance analysis of the safety of hydroxyapatite-derived products as bone graft extenders or substitutes for spine fusion. Eur Rev Med Pharmacol Sci 2015; 19 (19): 3548-3555.
3. Barbanti Brodano G, Mazzoni E, Tognon M, Griffoni C, Manfrini M. Human mesenchymal stem cells and biomaterials interaction: a promising synergy to improve spine fusion. Eur Spine J 2012; 21 (Suppl. 1): 3-9. doi: 10.1007/s00586-012-2233-z
4. Bolger C, Jones D, Czop S. Evaluation of an increased strut porosity silicate-substituted calcium phosphate, SiCaP EP, as a synthetic bone graft substitute in spinal fusion surgery: a prospective, open-label study. Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc 2019; 28 (7): 1733-1742. doi: 10.1007/s00586-019-05926-1
5. Bròdano GB, Giavaresi G, Lolli F et al. Hydroxyapatite-based biomaterials versus autologous bone graft in spinal fusion: an in vivo animal study. Spine 2014; 39 (11): E661-E668. doi: 10.1097/BRS.0000000000000311
6. Bruneau M, Nisolle JF, Gilliard C, Gustin T. Anterior cervical interbody fusion with hydroxyapatite graft and plate system. Neurosurg Focus 2001; 10 (4): E8. doi: 10.3171/foc.2001.10.4.9
7. Chang KY, Hsu WK. Spinal biologics in minimally invasive lumbar surgery. Minim Invasive Surg 2018; 5230350. doi: 10.1155/2018/5230350
8. Chau AMT, Mobbs RJ. Bone graft substitutes in anterior cervical discectomy and fusion. Eur Spine J 2009; 18 (4): 449-464. doi: 10.1007/s00586-008-0878-4
9. Chau AMT, Xu LL, Wong JH-Y, Mobbs RJ. Current status of bone graft options for anterior interbody fusion of the cervical and lumbar spine. Neurosurg Rev 2014; 37 (1): 23-37. doi: 10.1007/s10143-013-0483-9
10. Ciocca L, Donati D, Fantini M et al. CAD-CAM-generated hydroxyapatite scaffold to replace the mandibular condyle in sheep: preliminary results. J Biomater Appl 2013; 28 (2): 207-218. doi: 10.1177/0885328212443296
11. Coseo NM, Saldua N, Harrop J. Current use of biologic graft extenders for spinal fusion. J Neurosurg Sci 2012; 56 (3): 203-207.
12. D'Souza M, Macdonald NA, Gendreau JL, Duddleston PJ, Feng AY, Ho AL. Graft materials and biologics for spinal interbody fusion. Biomedicines 2019; 7 (4): 75 doi: 10.3390/biomedicines7040075
13. Dutta SR, Passi D, Singh P, Bhuibhar A. Ceramic and non-ceramic hydroxyapatite as a bone graft material: a brief review. Ir J Med Sci 2015; 184 (1): 101-106. doi: 10.1007/s11845-014-1199-8
14. Gupta A, Kukkar N, Sharif K, Main BJ, Albers CE, El-Amin III SF. Bone graft substitutes for spine fusion: a brief review. World J Orthop 2015; 6 (6): 449-456. doi: 10.5312/wjo.v6.i6.449
15. Kim P, Wakai S, Matsuo S, Moriyama T, Kirino T. Bisegmental cervical interbody fusion using hydroxy-apatite implants: surgical results and long-term observation in 70 cases. J Neurosurg 1998; 88 (1): 21-27. doi: 10.3171/jns.1998.88.1.0021
16. Kurien T, Pearson RG, Scammell BE. Bone graft substitutes currently available in orthopaedic practice: the evidence for their use. Bone Jt J 2013; 95-B (5): 583-597. doi: 10.1302/0301-620X.95B5.30286
17. Landi E, Logroscino G, Proietti L, Tampieri A, Sandri M, Sprio S. Biomimetic Mg-substituted hydroxyapatite: from synthesis to in vivo behaviour. J Mater Sci Mater Med 2008; 19 (1): 239-247. doi: 10.1007/s10856-006-0032-y
18. Manfrini M, Di Bona C, Canella A, et al. Mesenchymal stem cells from patients to assay bone graft substitutes. J Cell Physiol 2013; 228 (6): 1229-1237. doi: 10.1002/jcp.24276
19. Mashhadinezhad H, Samini F, Zare R. Comparison of outcomes and safety of using hydroxyapatite granules as a substitute for autograft in cervical cages for anterior cervical discectomy and interbody fusion. Arch Bone Jt Surg 2014; 2 (1): 37-42.
20. McConnell JR, Freeman BJC, Debnath UK, Grevitt MP, Prince HG, Webb JK. A prospective randomized comparison of coralline hydroxyapatite with autograft in cervical interbody fusion. Spine 2003; 28 (4): 317-323. doi: 10.1097/01.BRS.0000048503.51956.E1
21. Mobbs RJ, Phan K, Malham G, Seex K, Rao PJ. Lumbar interbody fusion: techniques, indications and comparison of interbody fusion options including PLIF, TLIF, MI-TLIF, OLIF/ATP, LLIF and ALIF. J Spine Surg 2015; 1 (1): 2-18. doi: 10.3978/j.issn.2414-469X.2015.10.05
22. Mokawem M, Katzouraki G, Harman CL, Lee R. Lumbar interbody fusion rates with 3D-printed lamellar titanium cages using a silicate-substituted calcium phosphate bone graft. J Clin Neurosci Off J Neurosurg Soc Australas 2019; 68: 134-139. doi: 10.1016/j.jocn.2019.07.011
23. Nandi SK, Roy S, Mukherjee P, Kundu B, De DK, Basu D. Orthopaedic applications of bone graft & graft substitutes: a review. Indian J Med Res 2010; 132: 15-30.
24. Pola E, Nasto LA, Tampieri A, et al. Bioplasty for vertebral fractures: preliminary results of a pre-clinical study on goats using autologous modified skin fibroblasts. Int J Immunopathol Pharmacol 2011; 24 (1 Suppl. 2): 139.
25. Rawlinson JN. Morbidity after anterior cervical

decompression and fusion. The influence of the donor site on recovery, and the results of a trial of surgibone compared to autologous bone. Acta Neurochir 1994; 131 (1-2): 106-118. doi: 10.1007/BF01401460

26. Samartzis D, Shen FH, Goldberg EJ, An HS. Is autograft the gold standard in achieving radiographic fusion in one-level anterior cervical discectomy and fusion with rigid anterior plate fixation? Spine 2005; 30 (15): 1756-1761. doi: 10.1097/01.brs.0000172148.86756.ce
27. Sartori M, Giavaresi G, Tschon M, et al. Long-term in vivo experimental investigations on magnesium doped hydroxyapatite bone substitutes. J Mater Sci Mater Med 2014; 25 (6): 1495-1504. doi: 10.1007/s10856-014-5177-5
28. Silber JS, Anderson DG, Daffner SD, et al. Donor site morbidity after anterior iliac crest bone harvest for single-level anterior cervical discectomy and fusion. Spine 2003; 28 (2): 134-139. doi: 10.1097/01.BRS.0000041587.55176.67
29. Suetsuna F, Yokoyama T, Kenuka E, Harata S. Anterior cervical fusion using porous hydroxyapatite ceramics for cervical disc herniation. a two-year follow-up. Spine J Off J North Am Spine Soc 2001; 1 (5): 348-357. doi: 10.1016/s1529-9430(01)00057-2
30. Thalgott JS, Giuffre JM, Klezl Z, Timlin M. Anterior lumbar interbody fusion with titanium mesh cages, coralline hydroxyapatite, and demineralized bone matrix as part of a circumferential fusion. Spine J Off J North Am Spine Soc 2002; 2 (1): 63-69.
31. Wigfield CC, Nelson RJ. Nonautologous interbody fusion materials in cervical spine surgery: how strong is the evidence to justify their use? Spine 2001; 26 (6): 687-694. doi: 10.1097/00007632-200103150-00027

Disclosure. *A. Nataloni and V. Canella are fully employed at Fin-Ceramica Faenza S.p.A. M. Sandri is fully employed at CNR-ISTEC, Faenza. The other Authors declare they have no financial or other conflict of interests in relation to this research and its publication.*

Review

Comparative studies on bone graft alternatives for common spine fusion procedures and focus on bioceramics

G. BARBANTI BRODANO*, B. ZANOTTI**

* *Department of Oncological and Degenerative Spine Surgery, IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy*
** *Neurosurgery Unit, Neuroscience Department, "C. Poma" Hospital, Mantua, Italy*

SUMMARY: *To improve a solid spinal fusion while avoiding morbidity of autograft harvesting procedures, numerous alternatives have been investigated during years, among which allograft, demineralized bone matrix, cell-based therapies and growth factors (i.e. bone morphogenetic proteins, platelet concentrates) and ceramic-based biomaterials. Even though all these approaches being potentially able to improve the outcomes of spinal fusion procedures, most of them have not been yet validated by evidence-based clinical results, thus resulting as poorly advisable for clinical use, furthermore particularly expensive. Here, we give an overview of the current clinical evidences for bone graft alternatives for spine surgery procedures. We will also evaluate the pros and cons of their use and briefly review the more relevant literature currently published.*

KEY WORDS: *Bioceramics, Spine surgery, Synthetic bone grafts.*

INTRODUCTION

Spinal fusion is one of the most common surgical procedures for treating conditions of the spine including deformity, trauma, degenerative disc disease and spondylolisthesis, where removal of the damaged anatomical structure is required[25].

Removal of such tissue results in mechanical instability of the spine, whereas the main goal of spinal fusion surgery is to fuse two or more vertebras, by inducing bone growth in-between such segments. Its use has dramatically increased over the last decades[12], with impacts on health care systems and on patients' quality of life.

Various techniques have been reported for the achievement of an adequate bone healing and solid fusion, including different surgical approaches, graft materials used and instrumentation method. However, despite numerous advances in spinal fusion procedures, pseudo arthrosis still occurs in about 25-35% of cases[32].

Additionally, patient- and treatment-associated factors, such as age, osteoporosis, number of levels treated, use of instrumentation or interbody grafts and surgical approach, influence the success of fusion[28,34]. As result, the research on new techniques to increase the success of spinal fusion and reduce pseudo arthrosis is strongly increasing.

Among the possibilities, ICBG and local autograft (spinous processes, laminae, facets, ribs) are still con-

Corrispondence: Dr. Giovanni Barbanti Brodano, Department of Oncological and Degenerative Spine Surgery, IRCCS Rizzoli Orthopaedic Institute, via Giulio Cesare Pupilli 1, 40136 Bologna (BO), e-mail: giovanni@barbantibrodano.com
Progress in Neurosciences 2021; 6 (1-4): 43-51. *ISSN: 2240-5127.*

LIST OF ACRONYMS AND ABBREVIATIONS: **β-TCP** = Beta-TriCalcium Phosphate; **AIS** = Adolescent Idiopathic Scoliosis; **ALIF** = Anterior Lumbar Interbody Fusion; **BMA** = Bone Marrow Aspirate; **BMPs** = Bone Morphogenetic Proteins; **DBM** = Demineralized Bone Matrix; **FGF** = Fibroblast Growth Factors; **HA** = HydroxyApatite; **HIV** = Human Immunodeficiency Virus; **ICBG** = Iliac Crest Bone Graft; **IGF** = Insulin Growth Factor; **LIF** = Lumbar Interbody Fusion; **Mg** = Magnesium; **MHA** = Mg-enriched HydroxyApatite; **MSCs** = Mesenchymal Stem Cells; **PLIF** = Posterior Lumbar Interbody Fusion; **PLF** = Posterior Lumbar Fusion; **rhBMP-2** = recombinant human Bone Morphogenetic Protein-2; **SiCaP** = Silicate-substituted Calcium Phosphate; **TGF** = Transforming-Growth Factor; **TLIF** = Transforaminal Lumbar Interbody Fusion; **XLIF** = eXtreme Lateral Interbody Fusion.

sidered the "gold standard" choices for bone replacement, due to the osteoconductive and osteogenic properties[25,50].

ICBG is mainly composed of cancellous bone obtained from the inner table of the pelvis. This type of bone graft shows a trabecular structure and a large surface area, which allows to mesenchymal stem cells and bone forming growth factors to be incorporated, an easy revascularization and a rapid incorporation to the host site[49], together with no risks of host rejection or infections and excellent fusion rates. However, patient finite supply, donor-site morbidity, acute or persistent pain[14,26] and adverse events (i.e., haematoma and infections, pelvic fracture, nerve palsy) are some of the most common related drawbacks which can affect patient quality of life, occurring in as many as 25-30% of patients[24,53]. Other additional drawbacks are longer operative times, increased estimated blood loss and longer hospital stay, which can increase risk for life-threatening complications (i.e. infections, deep vein thrombosis). These aspects have led to an increase in the development and use of bone graft substitutes and biological agents to achieve fusion. Depending on their properties, bone grafts augment natural healing via osteoinductive, osteoconductive and/or osteogenic mechanisms, mimicking the main properties of the human bone[39].

In this perspective, the present review is to give an overview of the current best evidence for bone graft alternatives, mimicking natural bone, in spinal surgery, and to review the most relevant literature.

□ ALLOGRAFT

Allograft has been used as alternative to autologous bone, in order to reduce autograft-related drawbacks[19]. National trends point to decreased utilization of autograft (86 to 10%) with a reciprocal increase in allograft (14 to 59%) from 1998 to 2004[44].

Allograft can be collected from either living or non-living donors and must be processed within a bone tissue bank. It requires sterilization (gamma-irradiation), with detrimental effects on mechanical properties of bone and deactivation of proteins normally found in healthy bone. As such, it is osteoconductive and weakly osteoinductive (growth factors may still be present, depending on the processing). Concerns on the potential infective risks, despite the use of strong aseptic techniques for allograft sterilization, and potential risks of contaminations are possible, although nowadays with very low risk rates. Contaminants and pathologies that may be transferred include bacterial infections, malignancy, systemic disorders (autoimmune disease), and toxins[19]. In particular, HIV and Hepatitis C virus transmission have also been reported, with a risk rate of 1:1.6 million[29,37,41]. In spine surgery, only one case of HIV transmission has been reported. The limits of such transplants are costs, difficult procedure (tissue processing, harvesting), mechanical resistance (in freeze dried and irradiated), limited osteoinduction and risk of infection.

□ DEMINERALIZED BONE MATRIX

DBM is the result of allograft decalcification (residual calcium < 5 %). Introduced since the early 1980s, this technique allows for the production of collagen matrix, non-collagenous proteins and growth factors (i.e. BMP, IGF, TGF, FGF), which can replicate the three-dimensional architecture of bone, therefore promoting and assisting the host cell invasion[7,17,27,39,45,50]. Due to the presence of bone-forming growth factors (such as BMPs, IGF, TGF, FGF), DBM also features osteoinductive properties, which allow progenitor cells growth and differentiation, and the induction of vascularisation. Compared to autograft or allograft, DBM lacks of donor-site related morbidity and shows excellent handling properties. However, the lack of the mineral component is associated with low mechanical strength. Despite the presence of numerous literature data on DBM effectiveness in preclinical studies on posterolateral spinal fusion[9,38,42,58], there are still limited evidences and also variable results produced in clinical studies to support the use of DBM as a stand-alone bone substitute[22,52,57]. This variability might be due to the lack of standardized processes for production, such as the particle size[15], along with donor-related issues, which results, above all, in BMP-2 and BMP-7 variations among different commercially available lots, or even in different batches from the same manufacturer[3].

☐ SYNTHETIC BONE GRAFTS

The history of synthetic materials starts in the early ’900, when D.E. Robertson assayed a piece of cat’s bone and a piece of human bone for bone grafting into dogs. The microscopic analysis of implanted graft after 20 days showed that the space between graft and the living bone was filled with new cancellous bone. These early works made the premises for the development of the bone grafts[1].

When compared to allografts, autografts and xenografts, synthetic grafts ahow some advantages, such as the possibility of unlimited number/quantity, a safety profile without risks of disease transmission, pain limitation by elimination of some secondary surgical intervention.

As reported by Ficai et al.[2], synthetic bone grafts can be divided in four categories, according to their evolution period (Table 1).

First generation bone grafts evolved in the second part of 1900, having remarkable mechanical properties but no resorbable nor bioactive properties. These first-generation bone grafts had limited lifetime (usually less than 10-15 years) and needed to be extracted and surgically replaced. Second-generation bone grafts were at least bioresorbable or bioactive and they did not require to be replaced in time. The most representative biomaterials from this second generation are represented by calcium phosphates (especially hydroxyapatite and tricalcium phosphate), bioglasses, alumina, zirconia, etc.

Third-generation bone grafts have been developed as bioresorbable and bioactive with superior properties, strongly influenced by the nature of components, the composition and the morphology. The most representative biomaterials of the third-generation of bone grafting biomaterials are: (nano)hydroxyapatite/collagen, (nano)hydroxyapatite/collagen/hyaluronic acid, hydroxyapatite/poly-L-lactic acid, etc.

The fourth-generation bone graft biomaterials have been developed with similar features of the former generation, but with an improvement in presence of bony cells, growth factors, bone morphogenetic proteins etc. One of the most important features of new bone grafts is osteointegration, which is strictly related to the degree of porosity and pore size of the scaffold.

As natural bone is mainly made of hydroxyapatite and collagen, many synthetic bone grafts have developed as biomaterials made of hydroxyapatite, β-TCP and also (nano)hydroxyapatite/collagen composite for hard tissue repairing (Table 2).

Hydroxyapatite, a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, is a crystalline calcium phosphate that is also manufactured as a ceramic through a sintering process. Because of the apatite structure of bone tissue, synthetic apatites are the most widely studied of all CaP phases. Stoichiometric HA (Ca/P ratio = 1.67) is a mineral composed of calcium ions, phosphate ions, and hydroxyl groups.

Because of this close similarity with the mineralized

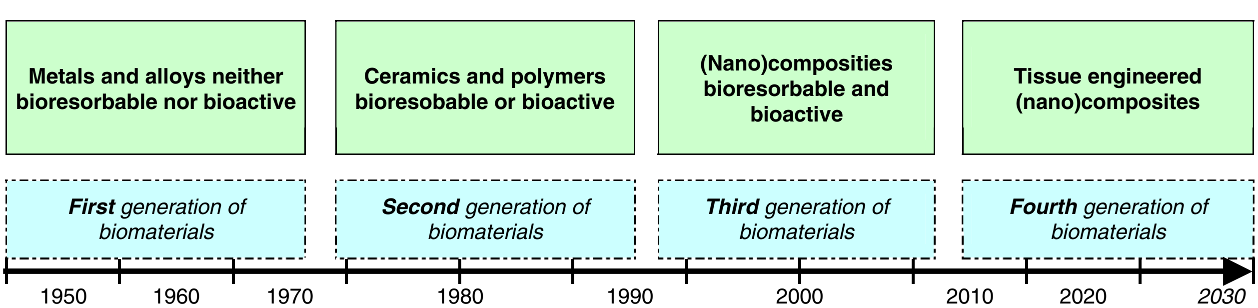


Table 1. Biomaterials evolution in the field of bone grafts (*modified from Ficai A et al., Andronescu E, Voicu G, Ficai D. Advances in collagen/hydroxyapatite composite materials*[2]).

Components	wt%
Mineral phase	
Hydroxyapatite	60-66
Carbonate (mostly as carbonated hydroxyapatite)	~ 4
Citrate	~ 0.9
Na^+	~ 0.7
Mg^{2+}	~ 0.5
Others	Traces
Organic phase	
Collagen	20-25
Non- collagenous proteins: (osteocalcin, osteonectin, osteopontin, sialloprotein, BMP)	2-3
Others	Traces
Water	8-9

Table 2. Composition of a healthy bone (*modified from Ficai A et al., Andronescu E, Voicu G, Ficai D. Advances in collagen/ hydroxyapatite composite materials*[2]). *Legend:* wt% = weight percentage.

phase of bone, HA shows high biomimetic properties, osteoconductive potential and excellent biocompatibility. The porous architecture of the HA substratum, with its macropore network and its micropore interconnections, induces rapid vascular and mesenchymal invasion and provides a specific cell flow. These cells can attach, proliferate, and finally differentiate into functional osteoblasts. The osteoconductive property of HA has made this material of relevant interest, being able to sustain new bone formation independently by the implantation site. As the new bone tissue incorporates the scaffold material, the scaffold itself should be biodegradable either through cellular events or as a result of the surrounding environment[43,54]. Ions substitution enhances HA unstability and biological activity, promoting rapid cell-mediated material resorption, new bone formation and remodelling[46]. As such, the design and development of a new generation of synthetic resorbable apatite substitutes has been stimulated to mimic some properties (chemical composition and three-dimensional architecture) of the biological phase to overcome most of the limitations typical of stoichiometric HA implants. Magnesium is certainly one of the most important bivalent ions associated with biological apatite, being one of the most abundant minerals in the human body and approximately 50% of Mg^{2+} is naturally present in the composition of bone tissue. Mg^{2+} enables the HA crystal cell structure to become unstable and more biologically active, promoting rapid cell-mediated material resorption, new bone formation and remodelling by cross-talking with progenitor cells at the molecular level[11,35,36]. Conversely, Mg^{2+} depletion affects all stages of skeletal metabolism, causing cessation of bone growth, decrease in osteoblastic activities, osteopenia and bone fragility. For all these reasons, Mg-HA has become of great interest in the development of effective bone substitutes[11,36]. Non-stoichiometric HA-based bone substitutes therefore provide an osteoconductive scaffold in which chemotactic cells and circulating proteins (e.g. mesenchymal stem cells, osteoinductive growth factors) can migrate and adhere, and into which progenitor cells can differentiate into functioning osteoblasts[48]. In vitro experiments revealed an active interaction between Mg-HA biomaterials and human MSCs, with an increased cell metabolic activity[6,40].

□ FINDINGS OF BIOCERAMICS IN ARTHRODESIS PROCESURES

In order to enhance or replace the function of autologous bone in spinal fusion it is relevant to use materials that are similar to autograft. Due to their intrinsic nature hydroxyapatite and / or tricalcium phosphate-based bioceramics have a chemico-physical characterization and a morphology more similar to bone than other materials and / or synthetic products.

Clinical studies carried out in spinal applications have shown good results on the use of hydroxyapatite in different formulations, as a stand-alone or mixed with autologous bone.

In the past, Korovessis et al.[33] showed HA bone bridging at 3 months post-op and radiological fusion 1 year postoperatively. The use of hydroxyapatite over the decorticated laminae, which represents a wide host area that offers much more bone chips from decortication, was followed by solid dorsal fusion within the expected time.

The use of HA combined with β-TCP and BMA was evaluated in a study by Bansal and colleagues[4] for posterolateral applications. At 12 months follow-up, radiological graft incorporation and fusion was evident in all the patients treated.

More recently, a meta-analysis of all randomized controlled trials published with a comparison on the effectiveness and safety of all available bone grafts in the different applications (PLF, PLIF, LIF, ALIF, TLIF, XLIF) has been carried out in 2019 by Feng and colleagues[16]. Among all the bone graft alternatives available, the Authors reported that synthetic ceramic materials (i.e. β-TCP, hydroxyapatite), used in combination with autograft local bone, show increased fusion rate, as compared to their use as stand-alone in all spinal applications. Additionally, they showed decreased frequency of complications, as compared to other alternatives (i.e. ICBG, rhBMP-2 or SiCaP).

Chang and colleagues[8] reported the same results when analyzing bone graft substitutes to be used in minimally invasive lumbar surgery; ceramics used as extenders to local autograft showed a fusion rate of about 86% in both posterior and anterior/lateral approaches, which is in line with what reported for open spine procedures[47].

In interbody fusion procedures, fusion rate and surgery-related factors affecting lumbar fusion were discussed by Formica and colleagues in their meta-analysis[18]. The Authors, who analized 67 studies (all belonging to level IV and III of evidence), reported an overall fusion of about 87% when ceramics (β-TCP, HA) where used. These results are in line with papers published years before by Thalgott[56] (2002), which reported a 94% of fusion in ALIF with HA and cages, or by Thaler[55] in 2013, which more recently showed good clinical outcomes at one-year follow-up in PLIF treated with ceramics and bone marrow aspirate. HA with β-TCP was also evaluated by Ransford[51] and Chou[10] in anterior cervical fusion and adolescent idiopathic scoliosis, which showed similar fusion rates as compared to the use of autologous bone, with fewer donor site complications.

In a recent study, Giorgi and colleagues evaluated the use of an HA-based biomaterial in form of chips mixed to autologous bone for PLF[20]. Results showed the incorporation of the HA chips into the fusion mass with formation of mature bony trabeculae and no sign of pseudo arthrosis. Moreover, new tissue formation in the interspaces between the vertebral bodies was evidenced.

In AIS, Delecrin et al.[13] performed a clinical study with 58 patients undergoing dorsal scoliosis correction of the spine column using instrumentation plus local bone graft combined with either iliac crest bone graft or hydroxyapatite and tricalcium phosphate. The results were assessed clinically at 4 years follow-up. Patients in the ceramic group showed lower average blood loss than those in the iliac graft group. They also were free from postoperative local complications in the iliac region, which were experienced by a significantly high proportion of patients belonging to the iliac graft group.

In degenerative scoliosis, a new generation hydroxyapatite biomaterial made of type I collagen fibrils with biomimetic MHA in aqueous media showed to promote bone regeneration and to assist new bone formation[21,30,31], in particular when applied in those procedures requiring huge amounts of bone graft available for the treatment of long spinal segments. Interestingly, a case-report recently published on the same biomaterial highlighted the osteointegrative properties of the device[23]. This is not common, as it almost never happen to review a patient previously operated at the spine. In this case, a bar broke and this led to the possibility of histologically examining a sample from where the bone graft biomaterial was placed, confirming the complete osteointegration of the device with no residuals in the biopsy fragment. A good bone tissue remodelling, due to ongoing osteogenic processes of the bone

In a prospective study published this year[5], twenty patients undergoing instrumented posterolateral spinal fusion using a biomimetic hydroxyapatite composite scaffold (enriched in magnesium and collagen) were radiographically evaluated at 12 months follow-up. The percentage of bony fusion was of about 95%, with no intra-operative or post-operative adverse events were recorded, providing clinical evidences of the fusion properties in posterolateral spinal fusion of the collagen-based HA scaffold employed.

All these results confirm that during years bone graft evolution allowed for the development of biomaterials able not only to provide three-dimensional structural support, but also, with chemical properties, to support bone ingrowth and tissue remodelling. Cumulative data from different materials used in spinal fusion are reported in Table 3.

Biomaterials have been reported, where possible, as commercial name.

In general, despite a generally low methodological quality across all the studies, overall results showed similar fusion rates among the different biomaterials, with no significant difference among each other. Even products with low clinical data available, or with recent history on the market, show good performance in terms of fusion rates achievable.

Product	Number of studies	Total patients	Number fused	Fusion rate (%)	Range (%)
Beta tricalcium phosphate (Vitoss; Stryker, Kalamazoo, USA)	7	345	319	92.5	85-100
Calcium sulfate (Osteoset; Wright Medical Technology, Memphis, USA)	6	353	306	86.7	45.5-92.4
Tricalcium phosphate/hydroxyapatite (BCP-BiCalPhos; Medtronic Sofamor Danek, Memphis, USA)	4	152	127	83.6	74.6-92.5
Coralline hydroxyapatite (Pro-Osteon 200, Pro-Osteon 500; Biomet, Warsaw, USA)	7	168	146	86,9	52.6-100
Type I collagen/hydroxyapatite (Healos; Depuy Synthes, Warsaw, USA)	5	97	83	85.6	77.3-95.5
Apatite-wollastonie-containing glass ceramic	2	36	36	100,0	NA
Dense hydroxyapatite block	1	26	25	96.2	NA
Synthetic hydroxyapatite (Bongros; Daewoong Bio Inc., Seoul, Korea)	1	45	39	86.7	NA
Silicate-substituted calcium phospahte	1	49	38	77.6	NA
Silikated hydroxyapatite (Actifuse; Baxter, Deerfield, USA)	1	39	31	79.5	NA
Hydroxyapatite-biocative glass (Chitra-HABg; Sree Chitra Tirunal, Trivandrum, India)	1	22	1	4.5	NA
Porous hydroxyapatite chips (Engipore; FinCeramica, Italy)	1	36	33	90.8	89.4-94.1
Type I collagen/hydroxyapatite (RegenOss; FinCeramica, Italy)	3	81	71	88.3	80.0-95.0

Table 3.Fusion rate by product.

☐ DISCUSSION

Bone harvest from iliac crest causes complications such as morbidity in the donor site, postoperative pain, hematoma, infections and increased blood loss with a frequency of 25-30% of patients; as h, the need to identify alternative sources to autologous bone has pushed research to the development of different biomaterials as bone grafts for achieving arthrodesis.
The increasingly frequent alternative to autologous bone graft is represented by bioceramic-based synthetic bone substitutes. Even within the limiting data reported in terms of statistical evidence, the literature highlights the excellent behavior of synthetic bone graft substitutes in comparison to autologous bone. In general, the resulting analyses evidenced that, despite a generally low methodological quality across all the studies, overall results demonstrated fusion rates and functional outcomes to be comparable between the considered bone graft extenders and iliac crest bone graft group, confirming the safety and efficacy of these materials in spinal fusion procedures. Among all the biomaterials available, hydroxyapatite in particular is recommended as bone graft substitute with close similarity to the human bone, when combined with rigid internal fixation for necessary support.

CONCLUSIONS

In conclusion, the use of ceramic derivatives as bone graft extenders is recommended to provide fusion. Despite the difficulties in evaluating the presence of new bone ingrowth with clinical tests (x-ray or CT scan may be unclear in determining whether the fusion masses represent new bone formation or the presence of an unfused ceramic mass), patients have shown good outcomes with the use of ceramics such as tri-calcium phosphate or hydroxyapatite. As such, their use is indicated as alternative to autologous and allograft bone, and as solution to avoid drawbacks related to withdrawal invasive procedures. In particular, hydroxyapatite is recommended as bone graft substitute when combined with rigid internal fixation for necessary support. Of note, all the studies reported the use of instrumentation as an adjunciton to the fusion procedure. In addition of all the osteoconductive bone graft extenders investigated, pooled quantitative analyses suggested that β-TCP and HA plus local autograft bone produced the most successful fusion rates, as compared to iliac crest bone graft.

In general, the resulting analyses highlight that, despite a generally low methodological quality across all the studies, overall results demonstrated fusion rates and functional outcomes to be comparable between the considered bone graft extenders and iliac crest bone graft group, confirming the safety and efficacy of these biomaterials in spinal fusion procedures.

REFERENCES

1. Attaf B (editor). Advances in composite materials for medicine and nanotechnology. IntechOpen, 2011. doi: 10.5772/1934.
2. Ficai A, Andronescu E, Voicu G, Ficai D. Advances in collagen/hydroxyapatite composite materials (chapter 1). In: B Attaf (editor): Advances in composite materials for medicine and nanotechnology. IntechOpen 2011: 3-32. Available from: www.intechopen.com/chapters/14592.
3. Bae HW, Zhao L, Kanim LE, Wong P, Delamarter RB, Dawson EG. Intervariability and intravariability of bone morphogenetic proteins in commercially available demineralized bone matrix products. Spine 2006; 31 (12): 1299-1306.
4. Bansal S, Chauhan V, Sharma S, Maheshwari R, Juyal A, Raghuvanshi S. Evaluation of hydroxyapatite and beta-tricalcium phosphate mixed with bone marrow aspirate as a bone graft substitute for posterolateral spinal fusion. Indian J Orthop 2009; 43 (3): 234-239.
5. Barbanera A., Vitali M., Nataloni A., Canella V. Clinical performance of a collagen-based hydroxyapatite bone graft substitute in procedures of spinal arthrodesis. Prog Neurosc 2020; 5 (1-4): 3-9.
6. Barbanti Brodano G, Mazzoni E, Tognon M, Griffoni C, Manfrini M. Human mesenchymal stem cells and biomaterials interaction: a promising synergy to improve spine fusion. Eur Spine J 2012; 21 (Suppl. 1): S3-9.
7. Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G, Lattanzi W, Logroscino G. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. J Mater Sci Mater Med. 2014; 25 (10): 2445-2461.
8. Chang KY, Hsu WK. Spinal biologics in minimally invasive lumbar surgery. Minim Invasive Surg 2018; 5230350.
9. Choi Y, Oldenburg FP, Sage L, Johnstone B, Yoo JU. A bridging demineralized bone implant facilitates posterolateral lumbar fusion in New Zealand white rabbits. Spine 2007; 32 (1): 36-41.
10. Chou YC, Chen DC, Hsieh WA, Chen WF, Yen PS, Harnod T, Chiou TL, Chang YL, Su CF, Lin SZ, Chen SY. Efficacy of anterior cervical fusion: comparison of titanium cages, polyetheretherketone (PEEK) cages and autogenous bone grafts. J Clin Neurosci 2008; 15 (11): 1240-1245.
11. Ciocca L, Donati D, Fantini M, Landi E, Piattelli A, Iezzi G, Tampieri A, Spadari A, Romagnoli N, Scotti R. CAD-CAM-generated hydroxyapatite scaffold to replace the mandibular condyle in sheep: preliminary results. J Biomater Appl 2013; 28 (2): 207-218.
12. Cortesi PA, Assietti R, Cuzzocrea F, Prestamburgo D, Pluderi M, Cozzolino P, Tito P, Vanelli R, Cecconi D, Borsa S, Cesana G, Mantovani LG. Epidemiologic and economic burden attributable to first spinal fusion surgery: Analysis from an Italian administrative database. Spine 2017; 42 (18): 1398-1404.
13. Delécrin J, Takahashi S, Gouin F, Passuti N. A synthetic porous ceramic as a bone graft substitute in the surgical management of scoliosis: a prospective, randomized study. Spine 2000; 25 (5): 563-569.
14. Dimar JR 2nd, Glassman SD, Burkus JK, Pryor PW, Hardacker JW, Carreon LY. Two-year fusion and clinical outcomes in 224 patients treated with a single-level instrumented posterolateral fusion with iliac crest bone graft. Spine J 2009; 9 (11): 880-885.
15. Dozza B, Lesci IG, Duchi S, Della Bella E, Martini L, Salamanna F, Falconi M, Cinotti S, Fini M, Lucarelli E, Donati D. When size matters: differences in demineralized bone matrix particles affect collagen structure, mesenchymal stem cell behavior, and osteogenic potential. J Biomed Mater Res A 2017; 105 (4): 1019-1033.
16. Feng JT, Yang XG, Wang F, He X, Hu YC. Efficacy and safety of bone substitutes in lumbar spinal fusion: a systematic review and network meta-analysis of randomized controlled trials. Eur Spine J 2020; 29 (6): 1261-1276.

17. Fischer CR, Cassilly R, Cantor W, Edusei E, Hammouri Q, Errico T. A systematic review of comparative studies on bone graft alternatives for common spine fusion procedures. Eur Spine J 2013; 22 (6): 1423-1435.

18. Formica M, Vallerga D, Zanirato A, Cavagnaro L, Basso M, Divano S, Mosconi L, Quarto E, Siri G, Felli L. Fusion rate and influence of surgery-related factors in lumbar interbody arthrodesis for degenerative spine diseases: a meta-analysis and systematic review. Musculoskelet Surg 2020; 104 (1): 1-15.

19. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury 2005; 36 (Suppl. 3): S20-27.

20. Giorgi P., Capitani D., Schirò G. Use of a novel porous hydroxyapatite bone graft substitute in postero-lateral and interbody spinal fusion: clinical and radiographic analysis at 4 year follow up. Prog Neurosc 2015; 3 (1-4): 117-124.

21. Giorgi P, Capitani D, Sprio S, Sandri M, Tampieri A, Canella V, Nataloni A, Schirò GR. A new bioinspired collagen-hydroxyapatite bone graft substitute in adult scoliosis surgery: results at 3-year follow-up. J Appl Biomater Funct Mater 2017; 15 (3): e262-e270.

22. Girardi FP, Cammisa FP Jr. The effect of bone graft extenders to enhance the performance of iliac crest bone grafts in instrumented lumbar spine fusion. Orthopedics 2003; 26 (5 Suppl.): s545-548.

23. Grigolo B, Dolzani P, Giannetti C, Tenucci M., Calvosa G. Use of a fully-resorbable, biomimetic composite hydroxyapatite as bone graft substitute for posterolateral spine fusion: a case report: A case report. Int. J Clin Exp Med 2016; 9 (11): 22458-22462.

24. Gruskay JA, Basques BA, Bohl DD, Webb ML, Grauer JN. Short-term adverse events, length of stay, and readmission after iliac crest bone graft for spinal fusion. Spine 2014; 39 (20): 1718-1724.

25. Gupta A, Kukkar N, Sharif K, Main BJ, Albers CE, El-Amin Iii SF. Bone graft substitutes for spine fusion: a brief review. World J Orthop 2015; 6 (6): 449-4456.

26. Howard JM, Glassman SD, Carreon LY. Posterior iliac crest pain after posterolateral fusion with or without iliac crest graft harvest. Spine J 2011; 11 (6): 534-537.

27. Hsu WK, Nickoli MS, Wang JC, Lieberman JR, An HS, Yoon ST, Youssef JA, Brodke DS, McCullough CM. Improving the clinical evidence of bone graft substitute technology in lumbar spine surgery. Global Spine J 2012; 2 (4): 239-248.

28. Irmola TM, Häkkinen A, Järvenpää S, Marttinen I, Vihtonen K, Neva M. Reoperation rates following instrumented lumbar spine fusion. Spine 2018; 43 (4): 295-301.

29. Khan SN, Cammisa FP Jr, Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology of bone grafting. J Am Acad Orthop Surg 2005; 13 (1): 77-86.

30. Kon E, Delcogliano M, Filardo G, Fini M, Giavaresi G, Francioli S, Martin I, Pressato D, Arcangeli E, Quarto R, Sandri M, Marcacci M. Orderly osteochondral regeneration in a sheep model using a novel nano-composite multilayered biomaterial. J Orthop Res 2010; 28 (1): 116-124.

31. Kon E, Filardo G, Delcogliano M, Fini M, Salamanna F, Giavaresi G, Martin I, Marcacci M. Platelet autologous growth factors decrease the osteochondral regeneration capability of a collagen-hydroxyapatite scaffold in a sheep model. BMC Musculoskelet Disord 2010;11: 220.

32. Kornblum MB, Fischgrund JS, Herkowitz HN, Abraham DA, Berkower DL, Ditkoff JS. Degenerative lumbar spondylolisthesis with spinal stenosis: a prospective long-term study comparing fusion and pseudarthrosis. Spine 2004; 29 (7): 726-733.

33. Korovessis P, Koureas G, Zacharatos S, Papazisis Z, Lambiris E. Correlative radiological, self-assessment and clinical analysis of evolution in instrumented dorsal and lateral fusion for degenerative lumbar spine disease. Autograft versus coralline hydroxyapatite. Eur Spine J 2005; 14 (7): 630-638.

34. Kurd M, Cohick S, Park A, Ahmadinia K, Lee J, An H. Fusion in degenerative spondylolisthesis: comparison of osteoconductive and osteoinductive bone graft substitutes. Eur Spine J 2015; 24 (5): 1066-1073.

35. Kurien T, Pearson RG, Scammell BE. Bone graft substitutes currently available in orthopaedic practice: the evidence for their use. Bone Joint J 2013; 95-B (5): 583-597.

36. Landi E, Logroscino G, Proietti L, Tampieri A, Sandri M, Sprio S. Biomimetic Mg-substituted hydroxyapatite: from synthesis to in vivo behaviour. J Mater Sci Mater Med 2008; 19 (1): 239-247.

37. Lomas R, Chandrasekar A, Board TN. Bone allograft in the U.K.: perceptions and realities. Hip Int 2013; 23 (5): 427-433.

38. Louis-Ugbo J, Murakami H, Kim HS, Minamide A, Boden SD. Evidence of osteoinduction by Grafton demineralized bone matrix in nonhuman primate spinal fusion. Spine 2004; 29 (4): 360-366.

39. Ludwig SC, Kowalski JM, Boden SD. Osteoinductive bone graft substitutes. Eur Spine J 2000; 9 (Suppl. 1): S119-125.

40. Manfrini M, Di Bona C, Canella A, Lucarelli E, Pellati A, D'Agostino A, Barbanti-Bròdano G, Tognon M. Mesenchymal stem cells from patients to assay bone graft substitutes. J Cell Physiol 2013; 228 (6): 1229-1237.

41. Manyalich M, Navarro A, Koller J, Loty B, de Guerra A, Cornu O, Vabels G, Fornasari PM, Costa AN, Siska I, Hirn M, Franz N, Miranda B, Kaminski A, Uhrynowska I, Van Baare J, Trias E, Fernández C, de By T, Poniatowski S, Carbonell R. European quality system for tissue banking. Transplant Proc 2009; 41 (6): 2035-2043.

42. Martin GJ Jr, Boden SD, Titus L, Scarborough NL. New formulations of demineralized bone matrix as a more effective graft alternative in experimental posterolateral lumbar spine arthrodesis. Spine 1999; 24 (7): 637-645.

43. Mastrogiacomo M, Papadimitropoulos A, Cedola A, Peyrin F, Giannoni P, Pearce SG, Alini M, Giannini C,

Guagliardi A, Cancedda R. Engineering of bone using bone marrow stromal cells and a silicon-stabilized tricalcium phosphate bioceramic: evidence for a coupling between bone formation and scaffold resorption. Biomaterials 2007; 28 (7): 1376-1384.

44. McGuire KJ, Harrast J, Herkowitz H, Weinstein JN. Geographic variation in the surgical treatment of degenerative cervical disc disease: American Board of Orthopedic Surgery Quality Improvement Initiative; part II candidates. Spine 2012; 37 (1): 57-66.

45. Miyazaki M, Tsumura H, Wang JC, Alanay A. An update on bone substitutes for spinal fusion. Eur Spine J 2009; 18 (6): 783-799.

46. Nandi SK, Roy S, Mukherjee P, Kundu B, De DK, Basu D. Orthopaedic applications of bone graft and graft substitutes: a review. Indian J Med Res 2010; 132: 15-30.

47. Nickoli MS, Hsu WK. Ceramic-based bone grafts as a bone grafts extender for lumbar spine arthrodesis: a systematic review. Global Spine J 2014; 4 (3): 211-216.

48. Paderni S, Terzi S, Amendola L. Major bone defect treatment with an osteoconductive bone substitute. Chir Organi Mov 2009; 93 (2): 89-96.

49. Pape HC, Evans A, Kobbe P. Autologous bone graft: properties and techniques. J Orthop Trauma 2010; 24 (Suppl. 1): S36-40.

50. Park JJ, Hershman SH, Kim YH. Updates in the use of bone grafts in the lumbar spine. Bull Hosp Jt Dis 2013; 71 (1): 39-48.

51. Ransford AO, Morley T, Edgar MA, Webb P, Passuti N, Chopin D, Morin C, Michel F, Garin C, Pries D. Synthetic porous ceramic compared with autograft in scoliosis surgery. A prospective, randomized study of 341 patients. J Bone Joint Surg Br 1998; 80 (1): 13-18.

52. Sassard WR, Eidman DK, Gray PM, Block JE, Russo R, Russell JL, Taboada EM. Augmenting local bone with Grafton demineralized bone matrix for posterolateral lumbar spine fusion: avoiding second site autologous bone harvest. Orthopedics 2000; 23 (10): 1059-1064.

53. Singh K, Phillips FM, Kuo E, Campbell M. A prospective, randomized, double-blind study of the efficacy of postoperative continuous local anesthetic infusion at the iliac crest bone graft site after posterior spinal arthrodesis: a minimum of 4-year follow-up. Spine 2007; 32 (25): 2790-2796.

54. Tampieri A, Celotti G, Landi E. From biomimetic apatites to biologically inspired composites. Anal Bioanal Chem 2005; 381 (3): 568-576.

55. Thaler M, Lechner R, Gstöttner M, Kobel C, Bach C. The use of beta-tricalcium phosphate and bone marrow aspirate as a bone graft substitute in posterior lumbar interbody fusion. Eur Spine J 2013; 22 (5): 1173-1182.

56. Thalgott JS, Giuffre JM, Klezl Z, Timlin M. Anterior lumbar interbody fusion with titanium mesh cages, coralline hydroxyapatite, and demineralized bone matrix as part of a circumferential fusion. Spine J 2002; 2 (1): 63-69.

57. Vaccaro AR, Stubbs HA, Block JE. Demineralized bone matrix composite grafting for posterolateral spinal fusion. Orthopedics 2007; 30 (7): 567-570.

58. Yee AJ, Bae HW, Friess D, Robbin M, Johnstone B, Yoo JU. Augmentation of rabbit posterolateral spondylodesis using a novel demineralized bone matrix-hyaluronan putty. Spine 2003; 28 (21): 2435-2440.

***Disclosure**. The Authors declare they have no financial or other conflict of interests in relation to this research ant its publication.*

Technical notes

□ Skin-sparing Neurosurgery

B. ZANOTTI

Neurosurgery Unit, Neuroscience Department, "C. Poma" Hospital, Mantua, Italy

SUMMARY: *With the exception of neuroradiosurgical treatment with Gamma Knife or other radiogenic sources, the surgical approach to the skull always involves skin damage. As the focus is intracranial, the post-surgical condition of the skin may not be a priority, but it is the most visible sign of neurosurgical intervention after healing. Furthermore, inadequate surgical planning and poor skin management can also compromise wound healing and lead to infectious, functional and/or aesthetic complications that can significantly affect the future life of the patient. This short communication highlights the use of the "Simpson" technique in the incision, with a view to maximising preservation of the vascular and lymphatic skin bed.*

KEY WORDS: *Craniotomy, Neurosurgery, Skin, Skin incision.*

The skin is what separates our insides from the outside. It is the visible part of us. The skin is an indispensable organ for both life and social wellbeing. It is at the nexus between medical science and sociology. This is fitting, since the skin originates from the ectoderm, the same embryonic germ layer that gives rise to both the central nervous system and the sense organs.

In fact, the skin can provide visual signal of nervous system alterations; think of Sturge-Weber syndrome, a rare congenital neurocutaneous disease characterized by capillary malformations on the face associated with neurological disease of varying degrees. Think also of von Recklinghausen's disease, with its different manifestations involving the skin, eye and nervous system. The list of such conditions would be long, and difficult to complete. Indeed, even common pathologies of the nervous system such as Parkinson's disease has implications for the skin, such as seborrhoea, accompanied by very particular odorous secretions.

In the modern world, the skin is a major source of social stigma, with discrimination based on fundamental skin functions such as melanin hyperpigmentation, which counteracts damage from solar radiation, and thermoregulation, which "civilized" people seek to disrupt via hair removal and antiperspirants.

The skin is also now a canvas for painting with make-up, piercings and tattoos, intended to display a state of mind (however fleeting), or to signal belonging to a particular social group, to instil respect or fear. In other latitudes and eras, skin modifications were markers of tribal identity-the context may change, but the substance does not.

Finally, the skin is the mirror of age. So much so, that people will do anything to stop the signs of aging; we invest far more energy and resources to appear younger than we do to counteract or slow down the decay of the mind and its substrate, the brain.

That being said, it is precisely in the field of human endeavour that seeks to restore the function of the latter, i.e., neurosurgery, that tends to ignore the importance of the skin. However, treating intracranial

Correspondence: Dr. Bruno Zanotti, Neurosurgery Unit, Neuroscience Department, "C. Poma" Hospital, 46100 Mantua, Italy, bruno.zanotti@rivistamedica.com
Progress in Neuroscience 2021; 6 (1-4): 53-56. *ISSN: 2240-5127*

pathologies often requires access through skin incision in cosmetic regions of the head and neck. Surgical success is typically measured in terms of the central part of the intervention, but the vast majority of complications tend to occur towards the end of the operation, that is, during closure of the incision(s) at the surgical site.

Some of the most difficult issues that a surgeon has to deal with are post-surgical wound complications, which will often involve the patient having to undergo further surgery; major complications such as infections of the meninges or central nervous system can even undermine all the positive outcomes of the primary intervention.

After surgery, tissue healing does not take place if the tissue flap does not receive an adequate supply of oxygen and nutrients necessary for tissue reconstruction. Hence, an adequate blood supply is essential for the optimal healing process, as are proper venous and lymphatic drainage. If the patient has already undergone previous cranial surgery, the surgeon should re-trace the old scars in order not to cause further aesthetic and/or functional tissue damage. In addition, he or she must be aware of what drugs the patient is taking, especially corticosteroids, and whether they have previously been subjected to radiotherapy, are suffering from a metabolic disease, such as diabetes, or practise habits that can negatively influence the healing process, like smoking.

When planning the intervention, the surgeon must place the utmost importance on the underlying arterial network; this, as well as the natural folds of the skin, must be considered when designing the incision. The scalp is one of the most highly vascularized tissues of the human body. It receives blood supply from both the internal and the external branches of the carotid artery. The four main branches of the external carotid artery are: the superficial temporal artery, the angular, occipital and posterior auricular arteries, and the supraorbital and supratrochlear arteries, which are terminal branches of the ophthalmic artery. The anterior portion of the scalp is supplied by the supratrochlear artery (also known as the frontal artery) and the supraorbital arteries. These vessels extend towards the vertex of the skull, and provide one of the anastomotic points between the internal and the external branches of the carotid system, joining the frontal sections of the superficial temporal artery. The superficial temporal artery replenishes the middle portion of the scalp, ascending anterior to the ear, and then dividing into the frontal branch, mentioned above, and the parietal branch, which supplies the temporal muscle and the parietal muscle of the scalp. There are anastomoses from the parietal branch of the superficial temporal artery to its counterpart on the opposite side, and to the homolateral occipital arteries, which supply the posterior portion of the scalp.

The scalp's venous drainage is divided into three components: the superficial, diploic and emissary. The superficial veins run along their arterial counterparts, while the diploic veins are located inside the diploe, the space between the internal and external layers of the skull. In the diploe, the veins are connected to the intracranial venous sinuses by means of the valveless emissary veins, which pass through tiny foramina in the skull. The four main groups of emissary veins are the posterior condyloid, mastoid, occipital, and parietal emissary veins. These veins provide bidirectional flow, typically from the outside to inside, but the direction can change in abnormal pathological states such as increased intracranial pressure. However, having veins without valves allows infections to spread from extracranial to intracranial sites, leading to septic thrombi.

Incisions that intersect a larger surface area can suffer from delayed wound healing, as such approaches carry a greater risk of compromising vascular supply. However, in large craniotomies, such as the pterional (frontotemporoparietal), this can be avoided by making an incision about 1 cm before the tragus, to avoid severing the main proximal trunk of the superficial temporal artery and the emerging branches of the facial nerve. A linear or "zig-zag", aka "Simpson", incision can then be made in order to allow tension to be dispersed through the incision. In this technique, the surgeon can also align the incision with the hairline, minimizing trauma to the follicle and thereby reducing the amount of hair loss at the site. The Simpson incision enables the surgeon to achieve excellent aesthetic results, not only in those with thick hair, but also on bald skulls. Especially in pterional flaps, it seems to represent an improvement as regards both the aesthetic outcome and patient satisfaction, as compared to the conventional curvilinear incision.

Then, while the intracranial surgery is underway, care must be taken to preserve the flap vitality, avoiding crushing or excessive traction, both possible causes of ischemia. Subgaleal drainage, if used, should also be as atraumatic as possible, and not create an obstacle to blood circulation. Direct visualization of

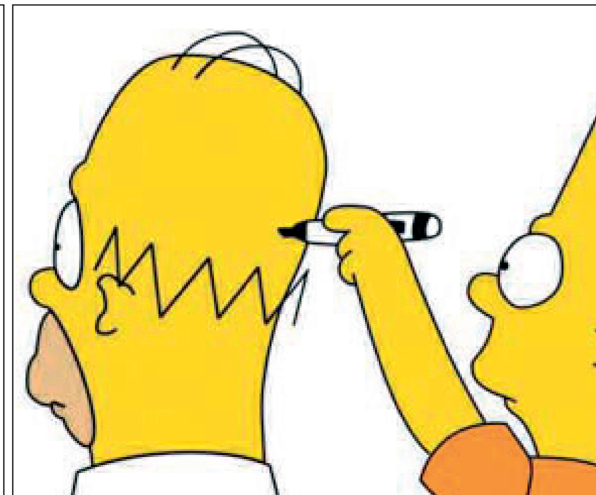


Figure 1. The Simpson family (famous American cartoon television series): Homer, Marge and their three children Bart, Lisa and little Maggie, with their hair, represent the paradigm of possible cranial approaches, with “zig-zag”, linear, “question mark” and bicoronal “stepwise” incisions.

Figure 2. Pterional incision via the “Simpson” technique.

the surgical flap and its complexion must be constant, and closely monitored in the days after surgery.
In order to optimize the outcome, the wound should be closed using intradermal sutures or staples. It is vital to use appropriate bandages, avoiding excessive pressure on the flaps, in order to contain and eliminate possible complications such as infections, ischemia and/or skin decubitus. Indeed, superficial infections that invade the central nervous system, among which meningitis, cerebritis, encephalitis, ventriculitis, abscess and empyema, may be fatal if not detected and treated quickly.
Neurosurgical wound healing competes with cerebral and systemic energy consumption, and can deprive the skin wound of the nutrition it needs for healing. While the human brain accounts for only 2% of the total body weight, it consumes more than 20% of its energy requirements, even in the absence of pathological lesions. After neurosurgery, particularly in patients with head trauma, the energy requirement is significantly greater, up to 32% or even 200%, depending on the severity of the injury.
Water supply must also be sufficient: dehydrated skin loses much of its elasticity, blood flow is compromised, and healing processes are interrupted. Another factor that surgeons do not always pay due attention to is the role of vitamins and minerals; vitamin C has long been known to be of fundamental importance in the formation of collagen, and also

plays a role in bone healing; selenium is used by fibroblasts; zinc aids in the debridement of devitalized tissue by macrophages; and copper is also essential for healing surgical wounds in tissues. However, nutrition is not only vital for surgical wound healing, it is also of fundamental importance for the area of the brain affected by surgery to treat the underlying pathology.

It is important to bear in mind, too, that wound healing can also be compromised by the pharmacological effects of some antibiotics, such as tetracycline and erythromycin, which inhibit leukocyte chemotaxis, and gentamicin, which delays wound re-epithelialization. Aspirin, ibuprofen and other non-steroidal anti-inflammatory drugs inhibit the inflammatory phase of wound healing and are platelet anti-aggregants. As a result, these medications can predispose patients to postoperative bleeding. In addition to inhibiting wound healing, non-steroidal anti-inflammatory drugs impede osteosynthesis by counteracting bone arthrodesis in spinal fusion procedures.

In the post-operative period, chemotherapy, radiotherapy and monoclonal antibodies can all inhibit and/or delay surgical wound healing, and, last but not least, the surgeon should also counsel the patient against the use of shampoos and cosmetics that can adversely affect the healing phase.

In short, although the neurosurgeon's target is undoubtedly the treatment of intracranial injury, a good neurosurgeon will try to "save the skin" of their patient, in the most literal sense of the term.

☐ REFERENCES

1. Berry JAD, Miulli DE, Lam B, Elia C, Minasian J, Podkovik S, Wacker MRS. The neurosurgical wound and factors that can affect cosmetic, functional, and neurological outcomes. Int Wound J 2019; 16 (1): 71-78.
2. Minami N, Kimura T, Uda T, Ochiai C, Kohmura E, Morita A. Effectiveness of zigzag Incision and 1.5-Layer method for frontotemporal craniotomy. Surg Neurol Int 2014; 5: 69. doi: 10.4103/2152-7806.132562.
3. Zanotti B, Verlicchi A. Craniectomie decompressive. In: B. Zanotti, A. Verlicchi, P.C. Parodi (editors): Cranioplastica terapeutica. Proceedings SNO: Master sulle Cranioplastiche terapeutiche custom made - LII Congresso Nazionale SNO, 11 maggio 2012, Roma. new Magazine edizioni, Trento: 37-46.

HISTORY OF NEUROSCIENCE

Original article

Ilya Metchnikoff: a Nobel Prize for phagocytosis

L. STERPELLONE

Clinical Pathologist
Science Communicator

SUMMARY: *This is an outline of the life, as both a scientist and a man, of the great Russian scientist Ilya Metchnikoff. He was awarded a Nobel Prize in 1908 for his research into immunology (cell-mediated immunity), which he summarized in his 1901 book Immunité, in which, going against the grain, he promoted the cellular (not humoral) theory of immune mechanisms. His range of knowledge was, however, eclectic, and he can also be considered the promoter of the widespread use in the West of soured milk (yoghurt) as a source of longevity. Outspoken against the Tsar, he found himself having to flee from his homeland. He moved to Messina (Italy), a city where he would make his famous discoveries on phagocytosis.*

KEY WORDS: *History of Medicine, Nobel Prize, Phagocytosis.*

THE DATES THAT MATTER

Ilya Metchnikoff was born in Kharkov (Russia) on 16 May 1845. After studying biology under R.A. Kölliker, he went to Paris, then to the Naples biological research institute, where he conducted systematic research into the development of germ layers in invertebrate embryos. However, he had to leave that city due to a cholera epidemic, going first to Germany and then returning to Russia.

From 1870 to 1882, he taught at the University of Odessa, but was forced to resign due to his anti-Tsarist activity. He moved to Messina, where he made his great discovery, phagocytosis, which earned him the 1908 Nobel Prize for Medicine (with Paul Ehrlich). Six years later he was called to Paris as Director of the Pasteur Institute, inaugurated in that year.

Still very young, at Luckart's laboratory in Giessen (Germany) Metchnikoff made his first major discovery, observing the alternation of generations (gamic and agamic) in nematodes. He also studied intracellular digestion in helminths in depth, glimpsing those mechanisms of incorporation and demolition that would lead him to the concept of phagocytosis, also inspired by the fact that in some metazoa *the processes of digestion and defence* are inextricably linked to each other.

After a bout of depression (during which he turned to morphine) following the untimely death of his first wife, he took a research trip to the Eurasian steppes; and on the basis of his observations he concluded that, compared to Caucasians, the Mongols' evolution had been interrupted; he ascribed the longevity of the Kalmyks to their diet, which mainly consisted of fermented milk.

He then suffered another period of depression, in which he attempted suicide a second time by injecting himself with the relapsing fever spirochete. In 1901 he published his seminal work *Immunité*; this book outlined his theories in support of the cellular

Correspondence: Dr. Bruno Zanotti, Neurosurgery Unit, Neuroscience Department, "C. Poma" Hospital, 46100 Mantua, Italy, bruno.zanotti@rivistamedica.com
Progress in Neuroscience 2021; 6 (1-4): 57-61. *ISSN: 2240-5127*

Figure 1. Élie Metchnikoff standing in a laboratory. Engraved by H. Davidson (*The Century illustrated monthly magazine. v.79., 1909*).

(and not humoral) theory of immune mechanisms, which went very much against the prevailing wisdom of the time. Among his other major works were: *Ätiologie, Prophylaxe der Infektionskrankheiten* (1897), *Der Lehr von den Phagocyten un deren exprerimentelle Grindlagen* (1904), and *Bacteriotyherapie, vaccinatio et, sérotherapie* (1908).

No less interesting are his investigations on the phenomena that promote aging; he studied a large group of Bulgarian centenarians who subsisted mainly on soured milk, which he examined under a microscope. He discovered that its activity was due to the presence of a bacillus, which he fittingly named *Lactobacillus bulgaricus*. From that day on, his second wife Olga (who was also his biographer) had to prepare gallons of soured milk for her husband, who made excessive use of it. Collaborators and acquaintances began to imitate him, and soon the consumption of soured milk became a viral trend that quickly spread to Paris and throughout France, Europe and overseas. Small and large industries for the production of yoghurt arose across the world. These soon proved to be productive and lucrative, but Metchnikoff had no interest in profiting from his discovery.

☐ THE DISCOVERY OF PHAGOCYTES

The 1908 Nobel Prize for Medicine was a reward for the pioneering research into immunology that the Russian researcher had carried out years earlier in his

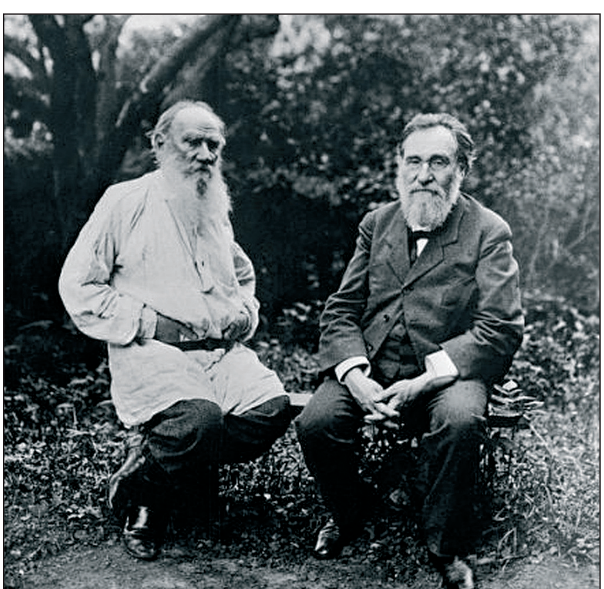


Figure 2. Leo Tolstoy (*left*) and Metchnikoff.

Figure 3. Mechnikoff and his wife Olga (approximately 1908).

small house on the Strait of Messina, where he had retired into voluntary exile to escape the Russian police; they were hunting him as a terrorist, allegedly involved in a conspiracy against the Tsar. He described his "Eureka!" moment as follows:

> *One day when the whole family had gone to a circus to see some extraordinary performing apes, I remained alone with my microscope, observing life in the mobile cells of a transparent star-fish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defence of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore to collect my thoughts.*
>
> *I said to myself that, if my supposition was true, a splinter introduced into the body of a star-fish larva, devoid of blood-vessels or of a nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done (O. Metchnikoff. Life of Eliè Metchnikoff, Constable & Co., London, 1921).*

The Russian scholar's predictions came true: near the thorn, the starfish larva appeared to fill with "mobile cells", that is to say leukocytes, ready to destroy it. He deduced that when an "intruder" enters the body, be it a thorn or a microbe, particular cells move towards it, surround it, and try to eat it.

It just so happened that in those days the great German pathologist Rudolf Virchow was passing through Messina; Metchnikoff explained to him his hypotheses on the defence mechanisms of organisms in detail, proudly showing him his preparation with the larva pierced by the rose thorn. He explained that in a starfish larva, devoid of a vascular system, leukocytes displayed the power to incorporate and destroy any microorganisms by virtue of their amoeboid movements, without the aid of blood flow to transport them to the site.

Virchow was profoundly impressed by this phenomenon, but he pointed out to his host that the prevailing opinion at that time among pathologists was literally the opposite-in their opinion, the "mobile cells", rather than destroying the bacteria, would incorporate them and disseminate them throughout the body. Leukocytes were the elements that *spread* the germs, and not, as Metchnikoff claimed, those which *destroyed* them!

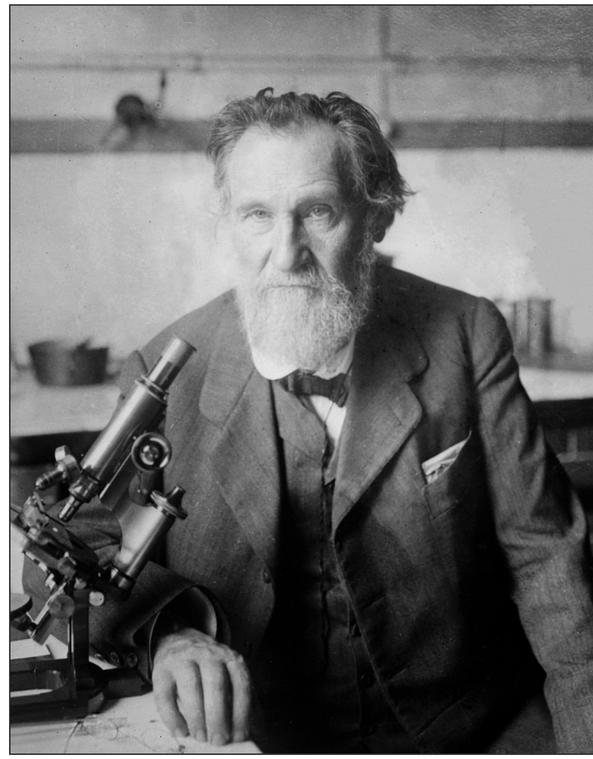


Figure 4. Professor Ilya Mechnikoff (between ca. 1910 and ca. 1915).

But Metchnikoff remained firm, and christened his mobile cells *Fresszellen*- "eating cells" in German, a rough but meaningful term. It would not be until later that his zoologist friend and lover of ancient Greek, C. Claus, coined the more elegant "*phagocyte*". The term *phagocytosis* naturally followed on from this, and was officially consecrated at the end of 1883 on the occasion of the 7th Congress of Russian Naturalists and Physicians in Odessa, where Metchnikoff presented his report *On the mesodermal phagocytes of some invertebrates*:

Figure 4. Memorial plaque for the discovery of phagocytosis by Mechnikov in 1882 at Messina (Italy).

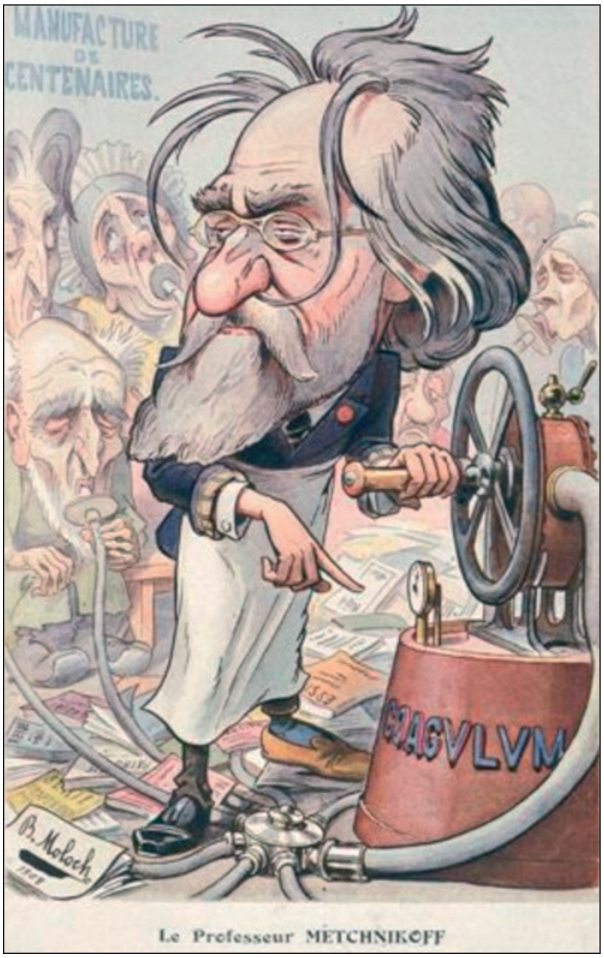


Figure 6. Caricature, spoofing Metchnikoff's enthusiasm for probiotics as a panacea, designed by Hector Moloch (signing B. Moloch) and published in the journal "*Chanteclair*" (2015).

> *I used the expression* phagocyte *to indicate various types of cells capable of capturing and digesting solid nutrients. The origin of the phenomenon of* phagocytosis *is to be found in the mesoderm, where there is a large number of amoeboid cells that devour foreign bodies, as well as dead or weakened autogenic elements. This power of leukocytes is well documented in pathology, although it has not yet been admitted that the incorporation of foreign bodies means food intake, or that the decomposition of ingested substances (such as blood cells) implies digestion. The results obtained in invertebrates and, especially the evidence that the sponge's amoeboid mesothelial cells play an important role in digestion (similar to that observed in* Bipinnaria, Phyllirhoe*, etc., in which these cells act as digestive organs), lead to the conclusion that intracellular digestion takes place in the mesodermal cells of vertebrates.*
> *To establish whether vertebrate phagocytes are also capable of devouring pathogenic bacteria, I caused artificial septicaemia in toads by subcutaneously injecting foetid blood. Leukocytes from infected animals have been shown to contain mobile and immobile pathogenic bacteria, including in vacuoles.*
> *I also found many of the pathogenic bacteria in the phagocytes of the spleen, which supports the theory accepted by many pathologists that leukocytes containing insoluble or poorly soluble material usually transport them to the spleen.*

Since 1865, when he was in Giessen, Metchnikoff had studied the intracellular digestion of a particular sandworm genus (*Fabricia*), relating this type of digestion to that observed in some protozoa: in this he glimpsed a sort of connection between lower and upper forms of animal life. Fifteen years later he published a paper on *intracellular digestion in coelenterates*, showing that some cells derived from the endoderm and mesoderm take up carmine granules suspended in water. However, he did not understand the mechanism behind this phenomenon.
In essence, according to Metchnikoff, the mechanisms of digestion and defence were essential "ingredients" of the body's defence process, which changes in relation to *evolution*. Thus, in the lower metazoan, defence and digestion were interchangeable attributes of the same type of cells; but with the development of the three germ layers (endoderm, mesoderm and ectoderm) in the higher zoological forms, it would be the mesoderm that would take on both functions. When digestive enzymes replaced intracellular digestion, the two functions became distinct, and the mesoderm retained only its defence function. Finally, in higher animals and humans, defence functions were carried out by even more diversified cells (the "phagocytes"), like leukocytes in blood, capillary endothelial cells, and large lymphatic tissue cells.
According to Metchnikoff, therefore, along the entire scale of invertebrate and vertebrate beings, phagocytosis is the characteristic aspect of inflammation (defence mechanism) that was already recognizable before the appearance of the vascular system. In fact, it began in the most primitive forms of life, as observed in single-celled beings, where, however, it had a purely nutritive function.

Obviously, Metchnikoff's research was part of the broader issue, namely the nature of the immune phenomenon (which he also called the "refractory condition"), which, supported by his own discoveries, he envisioned as follows:

> *As soon as it is born, the human being becomes the habitat of a very abundant microbial flora. The skin, mucous membranes and digestive system become pregnant with such flora, although a very limited part of this has been identified and described. The oral cavity, stomach, intestines and genital organs provide a rich* pabulum *for lower fungi and bacteria of various species. For a long time, it was believed that in the healthy individual all these microorganisms were harmless and sometimes even useful; and that when an infectious disease arises, a specific type of microorganism is* added *to the normal bacterial flora. Accurate bacteriological research has instead shown that, in reality, the varied vegetation of the healthy subject already includes representatives of various species of pathogenic microorganism. Apart from the diphtheria bacillus and cholera vibrio, which have been repeatedly found in the virulent form of perfectly healthy individuals, it has been shown that certain pathogenic microorganisms, such as* Pneumococcus*, Staphylococci, Streptococci and Bacillus coli, are always, or almost always, found among the microbial flora of the healthy subject.*
>
> *Such an observation necessarily led to the conclusion that, in addition to microorganisms, there is also a second factor of infectious disease-a "predisposition" or absence of immunity. An individual in which one of the above pathogenic species is present manifests a permanent or transient refractory state with regard to a specific microorganism; and when the cause of this immunity is lacking, the microorganism is activated and causes the specific disease.*
>
> *It is not necessary to multiply the number of such examples; they clearly demonstrate that in addition to the causes of disease that come from the outside world, there are other causes inherent in the organism itself. When these internal [defence] factors weaken, the disease arises; however, if they adequately resist the invasion, the organism is in a refractory condition and has immunity.*

In addition to phagocytosis, the Russian scholar would also turn his interest to the epidemiology of cholera, Typhoid fever and tuberculosis; but in the final years of his life, he would devote himself to a completely different problem: the best way to improve and prolong human life. This led to his theory of "orthobiosis", an optimistic conception of life and death based almost entirely on his own unshakable faith in Science; in his opinion this was the only way of pursuing, in the short term, the goal of "living a happy life until a peaceful death".

However, all his illusory hopes of seeing this dream come true were abruptly shattered in 1914 with the outbreak of World War I. The Russian scholar died two years later, without seeing its end, just in the days when the exhausting and bloody battle of the Somme was being fought in France.

☐ RECOMMEDED READING

1. Metchnikoff O. Life of Elie Metchnikoff 1845–1916. Constable & Co., London (UK), 1921.
2. Elie Metchnikoff (1845-1916), advocate of phagocytosis. JAMA 1968; 203 (2): 139-141.
3. Ezepchuk YV, Kolybo DV. Nobel laureate Ilya I. Metchnikoff (1845-1916). Life story and scientific heritage. Ukr Biochem J. 2016; 88 (6): 98-109.
4. Teti G, Biondo C, Beninati C. The phagocyte, Metchnikoff, and the foundation of immunology. Microbiol Spectrum 2016; 4 (2): MCHD-0009-2015. doi:10.1128/microbiolspec.MCHD-0009-2015
5. Cavaillon JM, Legout S. Centenary of the death of Elie Metchnikoff: a visionary and an outstanding team leader. Microbes Infect. 2016 Oct;18(10):577-594. doi: 10.1016/j.micinf.2016.05.008.
6. Cavaillon JM, Legout S. Duclaux, Chamberland, Roux, Grancher, and Metchnikoff: the five musketeers of Louis Pasteur. Genes Immun. 2019 May;20(5):344-356. doi: 10.1038/s41435-019-0064-1.
7. Gordon S. Elie Metchnikoff: father of natural immunity. Eur J Immunol 2008; 38 (12): 3257-3264.
8. Elie Metchnikoff (1845-1916), advocate of phagocytosis. JAMA. 1968; 203 (2): 139-141.

Acknowledgements. *This article is based on Dr. Bruno Zanotti's private correspondence with Dr. Luciano Sterpellone.*

Progress in Neuroscience

- An interdisciplinary journal -

PRINT ISSN 2240-5127
ON-LINE ISSN 2240-5135

PUBLISHER

EDITORE

NEW MAGAZINE S.R.L.
via dei Mille 69, 38122 Trento (TN), Italy

EXECUTIVE MANAGER

DIRETTORE RESPONSABILE

ANGELA VERLICCHI

EDITORIAL OFFICE, ADVERTISING

DIREZIONE, REDAZIONE, PUBBLICITÀ

new MAGAZINE edizioni, viale Rovereto 51, 38122 Trento (TN), Italy
www.progressneuroscience.com
e-mail: contact@progressneuroscience.com
www.newmagazine.it - e-mail: info@newmagazine.it

PRESS

STAMPA

Nuove Arti Grafiche, via dell'Òra del Garda 25, ZI settore A,
38121 Gardolo (TN), Italy

Authorized by the *Tribunale in Trento*, 11th May 2011 - Registration: *Registro della Stampa* n. 18 and *Registro degli Operatori di Comunicazione* (ROC) n. 3810 - *Quarterly*.

Each issue: 20 euro

Cover: Brain and spine in Leonardo da Vinci's anatomical drawings. From: Leonardo da Vinci. *The brain, cerebral ventricles and cranial nerves*, c.1508. Pen and ink, 19.2 x 13.5 cm. Schlossmuseum, Weimar (Germany). and *Diagram of the spine, nerves, vessels,* etc., and notes on the intercostal muscles, c. 1508-10 (Pen and ink over black chalk, 28.2 x 20.7 cm), Royal Collection Trust, Royal Library at Windsor Castle (United Kingdom).

NEW MAGAZINE EDIZIONI