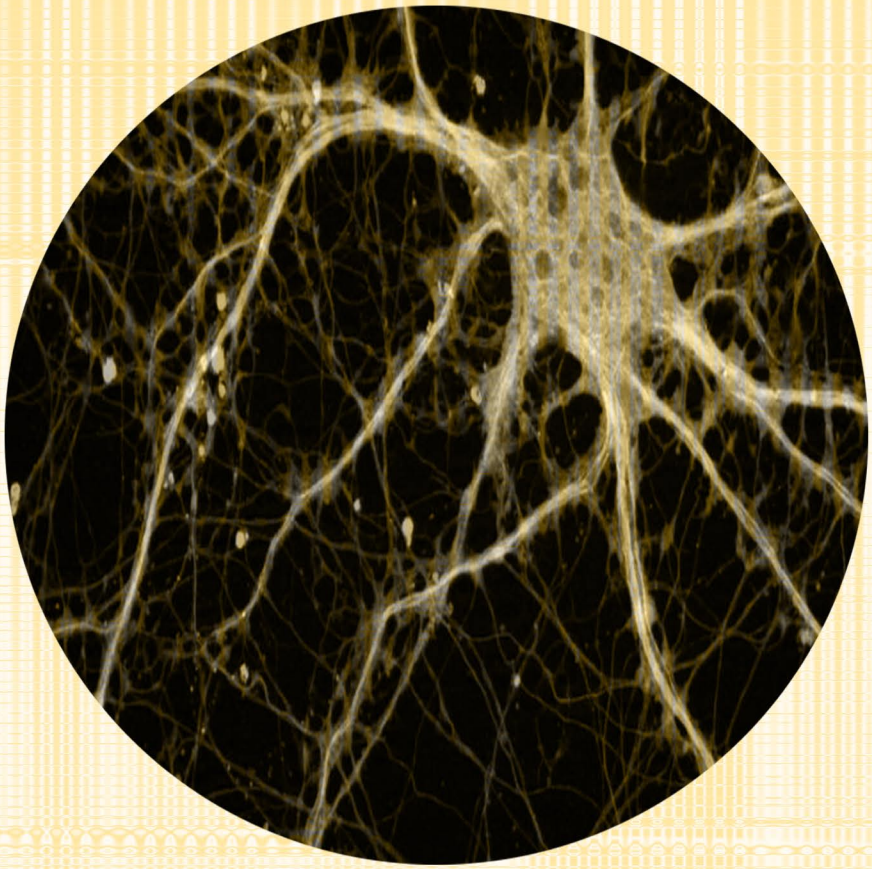
Openventio PUBLISHERS

ISSN 2377-1607

# NEURO

Open Journal

| December 2017 | Volume 4 | Issue 1 |





www.openventio.org

NEURO

Openventio PUBLISHERS

ISSN 2377-1607

Open Journal

# CONTENTS

NEURO

Openventio PUBLISHERS

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-e007

Editorial

*Corresponding author
**Gokul Krishna, PhD**
Department of Integrative Biology and Physiology
University of California Los Angeles (UCLA), 621 Charles E. Young Drive South, Los Angeles
CA 90095, USA
**E-mail:** gokul2411@gmail.com

***Volume 4 : Issue 1***
***Article Ref. #: 1000NOJ4e007***

***Article History***
**Received:** October 30th, 2017
**Accepted:** December 5th, 2017
**Published:** December 13th, 2017

***Citation***
Krishna G. Toxin-induced parkinson's disease models. *Neuro Open J*. 2017; 4(1): e1-e5. doi: 10.17140/NOJ-4-e007



# Toxin-Induced Parkinson's Disease Models

**Gokul Krishna, PhD***

*Department of Integrative Biology and Physiology, University of California, Los Angeles, CA 90095, USA*

The Topic "Toxin-Induced Parkinson's Disease Models' includes articles on experimental neurotoxic chemicals models of Parkinson's Disease (PD). The model toxicants with differing chemical structures recapitulate PD owning to their action on multiple molecular targets. These reports have provided with deeper understanding on the neurodegenerative events associated with the progressive disease. Several toxin-based models are developed in an attempt to experimentally mimic dopaminergic neurodegeneration, oxidative stress, cytoplasmic inclusions, proteasome dysfunction, altered protein trafficking, calcium overload and potentially mapping the events in the PD pathology. This spectrum includes research reports and reviews that discuss neurotoxin-based models (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-OHDA, rotenone, paraquat) that have greatly contributed to unravel the key mechanisms of neuronal cell death. In relevance to PD in humans, toxin-induced PD models have provided for observable behavioral deficits (motor and non-motor features). Moreover, the etiologic specific insights gained into the disease with the chemical modelling of PD has aided to screen/develop novel therapies.

PD is the most common progressive neurodegenerative condition affecting 1-2% of elderly population.[1] PD is characterized clinically by cardinal features involving resting tremor, rigidity and bradykinesia with loss of postural stability. The sporadic form of the PD involves progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) of the nigrostriatal system. Mutations in alpha-synuclein, parkin, ubiquitin carboxy-terminal hydrolase L-1 (UCHL1) and DJ-1 genes have been linked to familial forms of the disease.[2-4] Importantly, the PD pathology is characterized by the presence of fibrillary Lewy bodies and neurites, the intracytoplasmic proteinaceous inclusions containing neurofilament proteins. The occurrence of late-onset idiopathic form of the disease is likely due to gene mutation and environmental influence.[5] Nevertheless, there is a growing concern with the environmental factors particularly the chemicals to either induce PD or increase the disease risk.

Exposure to agricultural chemicals has been proposed to be a potential risk factor for the PD among the human population associated with farming, rural living and drinking contaminated well water.[6,7] Epidemiological data obtained from case reports, mortality reports, case-control studies relates to the possible link between chemical exposures and PD.[8-10] Also, *in vivo*, the chemical models possess certain limitations involving lack of specificity in their actions, systemic toxicity and failure to exactly model the non-motor features. Indeed, several reports suggest lack of an association between chemical exposure and PD development.[11-14] As will be discussed in the ensuing section, these findings are attributed to several factors involving exposure route, period (acute/chronic), and the relationship of chemical-induced neurotoxicity leading to development of disease. MPTP is highly lipophilic compound that is converted into active metabolite 1-methyl-4-phenylpyridinium (MPP+) by monoamine oxidase B to be taken up by the dopamine transporters (DATs). The metabolite translocates into mitochondrial matrix, inhibits complex I of the respiratory chain leading to adenosine triphosphate (ATP) depletion with increased formation of superoxide anion radical ($^{\bullet}O_{2^-}$).[15] Dysregulation in intracellular calcium homeostasis has also been proposed for neuronal degeneration with MPTP.[16] Additionally, MPTP treated has displayed enhanced neuronal oxidative and mitochondrial pathology.[17] MPTP intoxication has shown to enhance extracellular glutamate contributing to reactive oxygen species (ROS) release[18] leading to enhanced excitatory neurotransmitter activity in the basal ganglia. Importantly, MPTP promotes protein misfolding modifying

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-e007

chaperones such as alpha-synuclein by forming covalent adducts further leading to accumulation of aggregated proteins (Lewy bodies).[19]

6-hydroxydopamine (6-OHDA) continues to be a valuable PD model in rats[19] and is a structural analogue of biogenic amines-dopamine and noradrenaline. 6-OHDA injection into the ventrolateral caudate-putamen closely mimics human PD pathology through oxidative stress mechanism leading to nigrostriatal degeneration, inflammatory response and metabolic changes.[20,21] The molecule accumulates to generate reactive species to attack biological macromolecules by generating oxidative metabolites (reactive species and quinones) without Lewy body formation.[22] Several behavioral tests have been used to characterize the unilateral lesion associated with 6-OHDA injection.[23] The unilateral lesion has provided for quantitative assessment and behavioral deficits in rodents.[24]

Rotenone, a naturally occurring cytotoxic pesticide is highly lipophilic and potent inhibitor of mitochondrial complex I. Rotenone exposure reproduces many characteristic features of PD including nigrostriatal dopaminergic degeneration and formation of alpha-synuclein filamentous inclusions.[25] The pesticide treatment has shown to induce mitochondrial dysfunction with development of postural instability characteristic of PD.[26,27] Several studies in animals with rotenone exposure have implicated parkinsonism neurobehavioral abnormalities including locomotor defects,[28] depressive-like syndrome[29] and cognitive deficits[30]. Moreover, accumulating evidence points towards neuroprotective therapeutic intervention targeting vulnerable pathways involved in degenerative events following rotenone exposure.[31,32]

Paraquat (N,N-dimethyl-4-4-bipiridinium) is a member of the widely used bipyridyl herbicide with structural similarity to $MPP^+$ induces nigral dopaminergic neuronal loss and behavioral phenotype changes associated with human PD. The herbicide toxicity appears to be mediated through monocationic radical formation by NADPH:cytochrome P-450 reductase and NADH: ubiquinone oxidoreductase reduction of paraquat.[33] Reports indicate that paraquat crosses blood-brain barrier through the neutral amino acid transporter.[34] It is suggested that the pathological hallmarks of PD involving selective vulnerability of dopaminergic degeneration, a characteristic feature of paraquat neurotoxicity.[35] Studies have shown that paraquat-induced cell loss results from Bak-dependent pathway involving mitochondrial membrane permeabilization and subsequent activation of caspase-3. Apart from motor deficits, patients with PD often display neuropsychiatric pathology[39,40] and studies on animal behaviors related to affective-like state has been assessed with exposure to paraquat. Given the involvement of oxidative stress and dopaminergic cell loss in paraquat toxicity, various phytochemicals and other compounds have been studied to abrogate neurotoxic response.[41-45] Maneb (manganese ethylene-bis-dithiocarbamate), is a contact fungicide that synergistically interacts with paraquat to markedly reduce locomotor function and increased striatal terminals and nigral neuronal damage.[46] Such synergistic interactions have been considered for the role in PD etiology and are of interest since they reflect actual human exposures.[47]

Although, the toxic models reproducing PD neuropathological features have tremendously influenced our understanding of the disease, it is important to elucidate the causal relationship between toxin exposure and PD (that warrants quantitative data) since existence of methodological issues in establishing pesticide role in disease was suggested earlier.[48] However, pre-clinically the etiologic-specific neurotoxin PD models continue to investigate disease pathology owing to their merits to produce neuropathological features. Moreover, novel imaging modalities have provided better understanding of changes in neuronal activity responsible for behavioral outcome by neurotoxin challenge.[49] These advances have greatly aided in delineating mechanisms of PD neurodegeneration and potential therapeutics that may be applied. Further, environmental importance and likelihood of population exposure to toxicants must be taken into account when considering their use as model toxicants for PD.

## CONFLICTS OF INTEREST

The author declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## REFERENCES

1. McDonald WM, Richard IH, DeLong MR. Prevalence, etiology, and treatment of depression in Parkinson's disease. *Biol Psychiatry.* 2003; 54: 363-375. doi: 10.1016/S0006-3223(03)00530-4

2. Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci.* 2005; 28: 57-87. doi: 10.1146/annurev.neuro.28.061604.135718

3. Jankovic J. Parkinson's disease: Clinical features and diagnosis. *J Neurol Neurosurg Psychiatry.* 2008; 79: 368-376. doi: 10.1136/jnnp.2007.131045

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-e007

4. Mattila PM, Rinne JO, Helenius H, et al. Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. *Acta Neuropathol (Berl).* 2000; 100: 285-290. doi: 10.1007/s004019900168

5. Warner TT, Schapira AH. Genetic and environmental factors in the cause of Parkinson's disease. *Ann Neurol.* 2003; 53. doi: 10.1002/ana.10487

6. Peng J, Mao XO, Stevenson FF, Hsu M, Andersen JK. The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway. *J Biol Chem.* 2004; 279: 32626-32632. doi: 10.1074/jbc.M404596200

7. Alder T. Pesticides and Parkinson's disease: The legacy of contaminated well water. *Environ Health Perspect.* 2009: 117: A553.

8. Ballard PA, Tetrud JW, Langston JW. Permanent human parkinsonism due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Seven cases. *Neurology.* 1985; 35: 949-956.

9. Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *Am J Epidemol*. 2009; 169: 191-26. doi: 10.1093/aje/kwp006

10. Dhillon AS, Tarbutton GL, Levin JL, et al. Pesticide/environmental exposures and Parkinson's disease in East Texas. *J Agromedicine.* 2008; 13: 37-48. doi: 10.1080/10599240801986215

11. Firestone JA, Smith-Weller T, Franklin G, Swanson P, Longstreth WT Jr, Checkoway H. Pesticides and risk of Parkinson disease: A population-based case-control study. *Arch Neurol.* 2005; 62: 91-95. doi: 10.1001/archneur.62.1.91

12. Freire C, Koifman S. Pesticide exposure and Parkinson's disease: Epidemiological evidence of association. *Neurotoxicology.* 2012; 33: 947-971. doi: 10.1016/j.neuro.2012.05.011

13. Brown TP, Rumsby PC, Capleton AC, Rushton L, Levy LS. Pesticides and Parkinson's disease-is there a link? *Environ Health Perspect.* 2006; 114: 156-164. doi: 10.1289/ehp.8095

14. Searles Nielsen S, Hu SC, et al. Parkinsonism Signs and Symptoms in Agricultural Pesticide Handlers in Washington State. *J Agromedicine.* 2017; 22: 215-221. doi: 10.1080/1059924X.2017.1317684

15. Przedborski S, Vila M. The 1-Methyl-4-Phenyl-1, 2, 3, 6-Tetrahydropyridine Mouse Model. *Ann N Y Acad Sci.* 2003; 991: 189-198. doi: 10.1111/j.1749-6632.2003.tb07476.x

16. Surmeier DJ, Schumacker PT. Calcium, bioenergetics, and neuronal vulnerability in Parkinson's disease. *J Biol Chem.* 2013; 288: 10736-10741. doi: 10.1074/jbc.R112.410530

17. Hoang T, Choi DK, Nagai M, et al. Neuronal NOS and cyclooxygenase-2 contribute to DNA damage in a mouse model of Parkinson disease. *Free Radic Biol Med.* 2009; 47: 1049-1056. doi: 10.1016/j.freeradbiomed.2009.07.013

18. Lee DH, Kim CS, Lee YJ. Astaxanthin protects against MPTP/MPP+-induced mitochondrial dysfunction and ROS production in vivo and in vitro. *Food Chem Toxicol.* 2011; 49: 271-280. doi: 10.1016/j.fct.2010.10.029

19. Dehmer T, Lindenau J, Haid S, et al. Deficiency of inducible nitric oxide synthase protects against MPTP toxicity in vivo. *J Neurochem.* 2000; 74: 2213-2216. doi: 10.1046/j.1471-4159.2000.0742213.x

20. Blandini F, Armentero MT, Martignoni E. The 6-hydroxydopamine model: News from the past. *Parkinsonism Relat Disord.* 2008; 14: S124-S129. doi: 10.1016/j.parkreldis.2008.04.015

21. Guo S, Bezard E, Zhao B. Protective effect of green tea polyphenols on the SH-SY5Y cells against 6-OHDA induced apoptosis through ROS-NO pathway. *Free Radic Biol Med.* 2005; 39: 682-695. doi: 10.1016/j.freeradbiomed.2005.04.022

22. Iancu R, Mohapel P, Brundin P, Paul G. Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice. *Behav Brain Res.* 2005; 162: 1-10. doi: 10.1016/j.bbr.2005.02.023

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-e007

23. Betarbet R, Sherer TB, MacKenzie G, et al. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci.* 2000; 3: 1301-1306. doi: 10.1038/81834

24. Iancu R, Mohapel P, Brundin P, Paul G. Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice. *Behav Brain Res.* 2005; 162: 1-10. doi: 10.1016/j.bbr.2005.02.023

25. Sherer TB, Kim JH, Betarbet R, Greenamyre JT. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp Neurol.* 2003; 179: 9-16. doi: 10.1006/exnr.2002.8072

26. Jenner P. Parkinson's disease, pesticides and mitochondrial dysfunction. *Trends Neurosci.* 2001; 24: 245-246. doi: 10.1016/S0166-2236(00)01789-6

27. Cannon JR, Tapias V, Na HM, et al. A highly reproducible rotenone model of Parkinson's disease. *Neurobiol Dis.* 2009; 34: 279-290. doi: 10.1016/j.nbd.2009.01.016

28. Holinger GU, Lannuzel A, Khondiker ME, et al. The mitochondrial complex I inhibitor rotenone triggers a cerebral tauopathy. *J Neurochem.* 2005; 95: 930-939. doi: 10.1111/j.1471-4159.2005.03493.x

29. Johnson ME, Bobrovskaya L. An update on the rotenone models of Parkinson's disease: Their ability to reproduce the features of clinical disease and model gene-envirnment interactions. *Neurotoxicology.* 2015; 46: 101-116. doi: 10.1016/j.neuro.2014.12.002

30. Santiago RM, Barbieiro J, Lima MM, Dombrowski PA, Andreatini R, Vital MABF. Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. *Prog Neuropsychopharmacol Biol Psychiatry.* 2010; 34: 1104-1114. doi: 10.1016/j.pnpbp.2010.06.004

31. Kaur H, Chauhan S, Sandhir R. Protective effect of lycopene on oxidative stress and cognitive decline in rotenone induced model of Parkinson's disease. *Neurochem Res.* 2011; 36: 1435-1443. doi: 10.1007/s11064-011-0469-3

32. Gokul K, Muralidhara. Oral supplements of aqueous extract of tomato seeds alleviate motor abnormality, oxidative impairments and neurotoxicity induced by rotenone in mice: Relevance to Parkinson's disease. *Neurochem Res.* 2014; 39: 1382-1394. doi: 10.1007/s11064-014-1323-1

33. Anusha C, Sumathi T, Joseph LD. Protective role of apigenin on rotenone induced rat model of Parkinson's disease: Suppression of neuroinflammation and oxidative stress mediated apoptosis. *Chem Biol Interact.* 2017; 269: 67-79. doi: 10.1016/j.cbi.2017.03.016

34. Prakash J, Yadav SK, Chouhan S, Singh SP. Neuroprotective role of *Withania somnifera* root extract in Maneb-Paraquat induced mouse model of parkinsonism. *Neurochem Res.* 2013; 38: 972-980. doi: 10.1007/s11064-013-1005-4

35. Shimizu, K. Ohtaki K, Matsubara K, et al. Carrier-mediated processes in blood-brain barrier penetration and neural uptake of paraquat. *Brain Res.* 2001; 906: 135-142. doi: 10.1016/S0006-8993(01)02577-X

36. McCormack AL, Thiruchelvam M, Manning-Bog AB, et al. Environmental risk factors and Parkinson's disease: Selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. *Neurobiol Dis.* 2002; 10: 119-127. doi: 10.1006/nbdi.2002.0507

37. Fei Q, McCormack AL, Di Monte DA, Ethell DW. Paraquat neurotoxicity is mediated by a Bak-dependent mechanism. *J Biol Chem.* 2008; 283: 3357-3364. doi: 10.1074/jbc.M708451200

38. Dinis-Oliveira RJ, Remiao F, Carmo H, et al. Paraquat exposure as an etiological factor of Parkinson's disease. *Neurotoxicology.* 2006; 27: 1110-1122. doi: 10.1016/j.neuro.2006.05.012

39. Czerniczyniec A, Karadayian AG, Bustamante J, Cutrera RA, Lores-Arnaiz S. Paraquat induces behavioral changes and cortical and striatal mitochondrial dysfunction. *Free Radic Biol Med.* 2011; 51: 1428-1436. doi: 10.1016/j.freeradbiomed.2011.06.034

40. Litteljohn D, Mangano EN, Hayley S. Cyclooxygenase-2 deficiency modifies the neurochemical effects, motor impairment and co-morbid anxiety provoked by paraquat administration in mice. *Eur J Neurosci.* 2008; 28: 707-716. doi: 10.1111/j.1460-9568.2008.06371.x

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-e007

41. Nuti A, Ceravolo R, Piccinni A, et al. Psychiatric comorbidity in a population of Parkinson's disease patients. *Eur J Neurol.* 2004; 11: 315-320. doi: 10.1111/j.1468-1331.2004.00781.x

42. Hosamani R, Krishna G, Muralidhara. Standardized Bacopa monnieri extract ameliorates acute paraquat-induced oxidative stress, and neurotoxicity in prepubertal mice brain. *Nutr Neurosci.* 2016; 19: 434-446. doi: 10.1179/1476830514Y.0000000149

43. Singh M, Murthy V, Ramassamy C. Neuroprotective mechanisms of the standardized extract of Bacopa monniera in a paraquat/diquat-mediated acute toxicity. *Neurochem Int.* 2013; 62: 530-539. doi: 10.1016/j.neuint.2013.01.030

44. Mangano EN, Peters S, Litteljohn D, et al. Granulocyte macrophage-colony stimulating factor protects against substantia nigra dopaminergic cell loss in an environmental toxin model of Parkinson's disease. *Neurobiol Dis.* 2011; 43: 99-112. doi: 10.1016/j.nbd.2011.02.011

45. Chau KY, Korlipara LP, Cooper JM, Schapira AH. Protection against paraquat and A53T alpha-synuclein toxicity by cabergoline is partially mediated by dopamine receptors. *J Neurol Sci.* 2009; 278: 44-53. doi: 10.1016/j.nbd.2011.02.011

46. Somayajulu-Nitu M, Sandhu JK, Cohen J, et al. Paraquat induces oxidative stress, neuronal loss in substantia nigra region and parkinsonism in adult rats: Neuroprotection and amelioration of symptoms by water-soluble formulation of coenzyme Q10. *BMC Neurosci*. 2009; 10: 88. doi: 10.1186/1471-2202-10-88

47. Thiruchelvam M, Brockel BJ, Richfield EK, et al. Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: Environmental risk factors for Parkinson's disease? *Brain Res.* 2000; 873: 225-234. doi: 10.1016/S0006-8993(00)02496-3

48. Brown TP, Rumsby PC, Capleton AC, Rushton L, Levy LS. Pesticides and Parkinson's disease-Is there a link? *Environ Health Perspect.* 2006; 114: 156-164. doi: 10.1289/ehp.8095

49. Durand E, Petit O, Tremblay L, Zimmer C, et al. Social behavioral changes in MPTP-treated monkey model of Parkinson's disease. *Front Behav Neurosci.* 2015; 9: 42. doi: 10.3389/fnbeh.2015.00042

NEURO

Openventio PUBLISHERS

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-123

Research

*Corresponding author
**Massimiliano Farina, MD**
Director of Phlebolymphologic Diseases Centre
Monza Polyclinic
Via Amati 111
Monza (MB) 20900, Italy
**Tel.** +393356055219
**Fax:** +390396188077
**E-mail:** massimiliano.farina@angiologiamonza.com

***Volume 4 : Issue 1***
***Article Ref. #: 1000NOJ4123***

***Article History***
**Received:** December 22nd, 2016
**Accepted:** March 3rd, 2017
**Published:** March 3rd, 2017

***Citation***
Farina M, Novelli E, Pagani R. New criteria reduce inter-observer variability in chronic cerebrospinal venous insufficiency: A case control study. *Neuro Open J.* 2017; 4(1): 1-10. doi: 10.17140/NOJ-4-123



# New Criteria Reduce Inter-Observer Variability in Chronic Cerebrospinal Venous Insufficiency: A Case Control Study

**Massimiliano Farina, MD[1*]; Eugenio Novelli, PhD[2]; Raffaello Pagani, MD[3]**

*[1]Director of Phlebolymphologic Diseases Centre, Monza Polyclinic, Via Amati 111, Monza 20900, Italy*
*[2]Biostatistics Unit, San Gaudenzio Clinic (Monza Polyclinic Group), Novara 28100, Italy*
*[3]Angiology Unit, Villa Cimarosa Medical Centre, Milan 20144, Italy*

## ABSTRACT

**Background:** The pathophysiological mechanism defined "chronic cerebrospinal venous insufficiency" (CCSVI) diagnosed using Zamboni criteria has raised a heated debate about possible correlations with several neurological disorders, but also on subjectivity of these ultrasonographic criteria used for its diagnosis. Although in 2011 new criteria have been introduced to reduce the high inter-observer variability only two studies were conducted according to the new investigation protocol. Therefore, we wanted to verify the impact of the revised protocol and its ability to meet the demand for reduction of the high heterogeneity in inter-observer agreement.
**Patients and Methods:** Between June 2010 and June 2014, 1020 subjects (693 MS patients and 327 HCs) were prospectively screened for CCSVI by two investigators, blinded regarding the observed subject. After exclusion of discordant cases between the two examiners, 630 patients with clinically defined MS and 10 patients with CIS (clinically isolated syndrome) were matched by gender (male 38.7%, male/female 248/392) and age (mean age 44.0 years, range 18.5-77.0 years) with 315 HCs (male 43.2%, male/female 136/179-mean age 46.5 years, range 19.8-79.9 years).
**Results:** The prevalence of CCSVI in MS subjects before the introduction of the new ECD criteria (94.3%) was observed to be significantly reduced (83.4%) after their introduction ($p$<0.001). In MS patients, the strength of inter-observer agreement changed from moderate (k=0.532) to good (k=0.761) before/after the revision. B-mode analysis detected only 65.7% of valvular defects. Its diagnostic accuracy was 88.6% (95% CI: 84.0%-93.2%), with a sensitivity of 83.5% (95% CI: 75.2%-89.9%), a specificity of 96.0% (95% CI: 88.8%-99.2%), a positive predictive value of 96.8% (95% CI: 91.0%-99.3%) and a negative predictive value of 80.0% (95% CI: 70.3%-87.7%).
**Conclusions:** The new ECD criteria introduced by the revised protocol ensure, at this time, a substantial reduction of the inter-observer variability. Under this perspective, M-mode analysis is essential for its ability to identify the valvular abnormalities frequently not detectable by B-mode analysis.

**KEY WORDS:** CCSVI; Doppler ultrasound; Multiple sclerosis; Venous malformation; Venous reflux; Venous syndromes.

**ABBREVIATIONS:** CCSVI: Chronic cerebrospinal venous insufficiency; MS: Multiple Sclerosis; HCs: Healthy Controls; ECD: echo-colour-Doppler; IJVs: Internal Jugular Veins; VVs: Vertebral Veins; ISNVD: International Society for Neurovascular Disease; EDSS: Expanded Disability Status Scale.

## INTRODUCTION

In 2009, chronic cerebrospinal venous insufficiency (CCSVI) was described by Zamboni and

NEURO
Open Journal

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-123

colleagues as a component of the pathophysiology of multiple sclerosis (MS), immediately raising many questions about the cause of MS, and how it may be treated based on the theory of CCSVI, especially not to compromise patient safety during the verification of a research hypothesis.[1,2] Some authors have suggested that impaired extracranial venous drainage secondary to valvular defects and/or wall miopragia or extrinsic muscular compression, particularly in the internal jugular veins (IJVs), azygos and vertebral veins (VVs), would cause endothelial inflammation, suggesting a role for perivenous iron deposition in the autoimmune mechanisms responsible for this demyelinating disorder.[1,3,4] Likewise, dysfunctions of autonomic nervous system could also be involved by altering postural control of arterial inflow and venous outflow.[5] On the other side, some reports of scientific societies claim that literature data are insufficient to establish the importance of CCSVI as a major factor in MS pathogenesis. In the same way as a result of limited research would be questioned the effectiveness of the angioplasty procedure.[6] In this state of uncertainty, not comforted by conflicting MRI studies, was emphasized the need to establish the indication for surgery according to individual indications, after a detailed discussion between physician and patient.[7-9]

So far, the diagnosis of CCSVI has been obtained with echo-colour Doppler (ECD) by means of detecting at least two of the Zamboni criteria according to the revised protocol of the International Society for Neurovascular Disease (ISNVD) of 2011 (Table 1).[10] As recently reported in a meta-analysis by Zwischenberger, from 2005 through 2013, 13 studies evaluated the prevalence of CCSVI in 1141 patients with MS and 738 matched healthy controls (HCs).[11] After removing four outlying studies to improve homogeneity, the analysis of the remaining studies showed a significant correlation between CCSVI and MS (OR 1.885, $p<0.0001$). In another meta-analysis, Tsivgoulis identified 19 eligible studies involving 1250 MS patients and 899 HCs, showing a significant association (OR 8.35, $p<0.001$) between CCSVI and MS but considerable heterogeneity across the studies ($I^2=80.1\%$).[12] This fact has been mainly ascribed to the involvement of the authors in the new endovascular treatment of CCSVI. Indeed, a most conservative sensitivity analysis combining different exclusion criteria showed no association of CCSVI with MS (OR 1.35; 95% CI 0.62-2.93; $p=0.453$) without any heterogeneity ($I^2=0\%$). Based on these findings, some authors have claimed the suspension of angioplasty procedures of the extracranial veins.[13,14] Although, ECD studies are greatly influenced depending on the individual patients (different ages, differences in clinical forms and disability, physiological factors such as head position, hydration status and different degrees of cervical muscle relaxation and breathing) and operators, showing high inter-observer variability for untrained examiners,[15] only two studies have been conducted with the new ISNVD criteria.[16,17] In fact, apart from these only exceptions, all studies regarding CCSVI published after the revised protocol in 2011 continued to adopt the old criteria, affecting the results of the meta-analysis, because of the low agreement of the former criteria.[18-34] Also the two studies concerning the inter-observer variability have referred to the old protocol.[35-36] The aims of this study were to assess the overall prevalence of CCSVI in MS patients and in matched voluntary HCs observed between 2010 and 2014, to compare this prevalence, diagnosed by the old and new ECD criteria, in MS patients before and after the November 2011 criteria revision, and to evaluate if the new ECD criteria could reduce inter-observer variability.

## MATERIAL AND METHODS

### Patients and Controls

Between June 2010 and June 2014, 1020 subjects (693 MS patients and 327 HCs) were prospectively screened for CCSVI by

**Table 1.** Five Zamboni Criteria According to the Revised Protocol of the International Society for Neurovascular Disease.

| Criteria | Old | New |
|---|---|---|
| 1. | Reflux constantly present in IJVs and/or VVs with the head at 0° and +90° (flow reversal from its physiological direction for a duration of >0.88 s) | a. Bidirectional flow in one or both of the IJVs in both postures or bidirectional flow in one position with absence of flow in the other position<br>b. Reversal or bidirectional flow in one or both of VVs in both positions |
| 2. | Reflux in the DCVs (>0.5 s) | Bidirectional flow (or reflux) in the intracranial veins and sinuses (additional criterion) |
| 3. | High resolution B-mode evidence of proximal IJV stenoses (CSA of IJV in the supine position ≤0.3 $cm^2$) | a. Severe reduction of the CSA of IJV in the supine position <0.3 $cm^2$ which does not increase with Valsalva manoeuvre<br>b. Intraluminal defects (webs, septa or malformed valves) combined with hemodynamic changes (increased velocity, absence of flow, reflux/bidirectional flow, etc.). M-Mode analysis may clarify the presence of defective valves (mobile or not, slightly mobile). |
| 4. | Flow not Doppler detectable in the IJVs and/or VVs despite numerous deep inspirations, with the head positioned at 0° and +90° | Absence of Doppler signal in the IJV and/or the VV, even after deep inspiration, in both sitting and supine positions or in one posture but with bidirectional flow detected in the other position |
| 5. | Reverted postural control of the main cerebral venous outflow pathways (negative ΔCSA value) | A CSA of the IJV which is greater in the sitting position than in the lying position or appears almost unchanged despite change in posture |

CSA=cross sectional area; DCVs=deep cerebral veins; ΔCSA=obtained by subtracting the CSA measured in the supine from that in the sitting position; IJV=internal jugular vein; IJVs=internal jugular veins; VV=vertebral vein; VVs=vertebral veins.

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-123

**Table 2:** Demographic and Clinical Characteristics of MS Patients.

| Variable | n=640 |
|---|---|
| **Female gender** | 392 (61.3%) |
| **Age (years)** | 44.0±11.7 (18.5-77.0) |
| **Disease duration (years)** | 10.0±7.5 (0-40) |
| **Clinical subtypes** | |
| - CIS | 10 (1.6%) |
| - RRMS | 416 (65.0%) |
| - SPMS | 155 (24.2%) |
| - PPMS | 59 (9.2%) |
| **EDSS score** | 3.41±1.91 (0-9) |
| **VHISS score** | 5.27±2.02 (0-12) |
| **CCSVI score** | 2.73±0.94 (0-5) |
| **Number of positive criteria** | |
| - 0 | 14 (2.2%) |
| - 1 | 40 (6.3%) |
| - 2 | 171 (26.7%) |
| - 3 | 304 (47.5%) |
| - 4 | 99 (15.5%) |
| - 5 | 12 (1.9%) |
| **Year of examination** | |
| - 2010 | 70 (10.9%) |
| - 2011 | 419 (65.5%) |
| - 2012 | 73 (11.4%) |
| - 2013 | 62 (9.7%) |
| - 2014 | 16 (2.5%) |
| **Examination according to November 2011 ECD criteria revision** | |
| - old ECD criteria | 477 (74.5%) |
| - new ECD criteria | 163 (25.5%) |

CCSVI=chronic cerebrospinal venous insufficiency; EDSS=Expanded Disability Status Scale; MS=multiple sclerosis; PPMS=primary progressive multiple sclerosis; RRMS=relapsing remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis; VHISS=venous hemodynamic insufficiency severity

two experienced vascular sonographers by evaluating the presence of at least two of the Zamboni criteria. After exclusion of discordant cases between the two examiners, 630 patients with clinically defined MS (according to the 2010 revised McDonald diagnostic criteria)[37] and 10 patients with CIS (clinically isolated syndrome) were matched by gender (male 38.7%, male/female 248/392) and age (mean age 44.0 years, range 18.5-77.0 years) with 315 HCs (male 43.2%, male/female 136/179-mean age 46.5 years, range 19.8-79.9 years) who were students and technical and administrative staff from our hospital. An MS specialist evaluated all the subjects by means of physical and neurological examinations, assigning disability scores according to the Kurtzke scale expanded disability status scale (EDSS). Table 2 shows the MS patients' demographic and clinical characteristics. The investigations were performed using the standard ECD criteria for subjects examined before November 2011,whereas the new ultrasonographic criteria were used for subjects examined there after (Table 1).[1,10] The exclusion criteria from the study were previous head or neck surgery, neck swelling, severe heart disease, serious kidney and liver diseases, thrombosis of the jugular vein(s), jugular vein catheterisation, vasculitis, Behçet's syndrome, collagen diseases, congenital cerebral malformations and congenital vascular malformations. Our Ethical Committee (Local Health Authority, Monza Brianza, Italy) approved this case-control study, and all the participants provided written informed consent.

### Duplex Ultrasound Investigation

Two vascular sonographers who had undergone the same special training performed all the investigations. They were particularly experienced with venous diseases, and each one had performed approximately 10,000 ultrasound investigations per year over the past few years. Each operator recorded all the scans for subsequent reconstruction and morphological analysis. Thus, each of the two investigators, in a blind fashion regarding the observed subject, also assessed all the exams performed by the other operator. All discordant cases that emerged from this comparison were also excluded from the study. All evaluations were performed in the morning after adequate fluid intake during the 24 hours preceding the examination (500 ml upon waking before the exam) to avoid dehydration, as reported in many studies.[38] The subjects were first evaluated in the supine position (0°) and then in the upright sitting (90°) position, with head in a neutral position (0° midline) placed upon a small pillow (8 cm height) to reduce in supine positionthe tone of the neck musculature.[39] During the position changes, a resting condition (no voluntary muscle movements or contractions) was obtained with a proper electromechanical tiltable chair. All veins [both IJVs and VVs, deep cerebral veins (DCVs)] were evaluated in the laterocervical area of the neck and through the transtemporal and transoccipital windows, using a large amount of gel to assure perfect coupling of the transducer, according to the screen-

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-123

ing protocols for CCSVI with ultrasound and other studies.[10,40] In the evaluation of DCVs (vein of Rosenthal, vein of Galen, transverse sinus, straight sinus and internal cerebral vein) the problems related to a Doppler angle of insonation close to 90° were solved by using a multi-angle Doppler system such as the Quality Doppler Processing technology (QDP). QDP helps in detecting the blood flow direction within the cerebral veins. To-compute the cross-sectional area (CSA-mm$^2$) of IJVs, we used an ellipsoid or continuous trace method, referring to the greatest ellipse at the end-expiratory phase. All measurements obtained were repeated 3 times, and the average of the three measurements was used for comparison. After the revised protocol, all the subjects were submitted to M-mode analysis of the IJVs to better clarify the presence of valvular defects frequently not easily identifiable with B-mode analysis (Table 1).[16] B-Mode is a two-dimensional ultrasound image display composed of bright dots representing the ultrasound echoes. The brightness of each dot is determined by the amplitude of the returned echo signal. This allows for visualization and quantification of anatomical structures. The M-mode represents movement of structures over time having a good temporal resolution, so it is useful to study the movement of vessel walls and valves by detecting valve incompetence and abnormal structures. Initially a 2-D image is acquired and a single scan line is placed along the area of interest. The M-mode will then show how the structures intersected by that line move toward or away from the probe over time.Valve defects related to leaflets movement were classified as mobile, slightly mobile and not-mobile. For transcranial and extracranial scans, we used the same ECD unit (MyLabVinco, Esaote SpA, Florence, Italy) equipped with a linear and a phased array transducer probe, operating bandwidth 1-4 MHz (B-modes Frequencies, 2.0-2.5-3.3 MHz; Doppler Frequencies, 1.6-2.0-2.5 MHz) and 3-11 MHz (B-modes Frequencies, 3.5-5.0-6.6-10.0 MHz; Doppler Frequencies, 3.3-5.0 MHz). The device, supplied with software for automatic calculation of the CCSVI score and venous hemodynamic insufficiency severity score (VHISS), was upgraded in November 2011 according to the new revision of the ECD criteria.[10]

### Statistical Analysis

Continuous variables are described as the means and standard deviations, whereas counts and percentages are used to describe qualitative variables. Inter-observer agreement between the two sonographers was calculated with Cohen's k statistics. The degree of concordance was considered as follows: low ($0 \leq k \leq 0.4$), moderate ($0.4 < k \leq 0.6$), good ($0.6 < k \leq 0.8$), excellent ($0.8 < k \leq 1$). A frequency matching approach for gender and age was applied in this study. Correlations of CCSVI with clinical subtype, gender, age and disease duration were tested using $X^2$ statistics. The CCSVI exposure of cases and controls were compared to give estimates of the association between CCSVI and MS. Odds ratios (ORs) were calculated using direct computation from 2×2 tables to quantify how strongly the presence or absence of CCSVI is associated with the presence or absence of the neurological disease. Prevalence rates for each of the five Zamboni criteria were calculated. In detecting valvular defects, B-mode analysis was compared to M-Mode by the measures of diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value. In this comparison, the M-Mode analysis was considered as a gold standard for its capability to better clarify the presence of defective valves.

The statistical analyses were performed using SPSS software (version 14.0, SPSS Inc., Chicago, IL, USA). All 2-tailed *p*-values below 0.05 were considered statistically significant.

## RESULTS

The demographic and clinical characteristics of the MS patients are shown in Table 2 along with the year of the ECD examination. The patients belonged to four clinical subtypes: 416 (65.0%) were suffering from a relapsing-remitting (RR) clinical form, 155 (24.2%) from a secondary progressive (SP) form, and 59 (9.2%) from a primary progressive (PP) form and 10 (1.6%) from clinically isolated syndrome (CIS). Female gender was prevalent (61.3%), with a mean age of 44 years and a mean disease duration of 10 years. Due to the November 2011 ECD criteria revision, 74.5% of the patients were examined with the old ECD criteria and the remaining 25.5% were examined with the new criteria. After the blinded comparison of two sonographers 640/693 (92.4%) MS patients and 315/327 (96.3%) HCs were considered suitable for the study. Among these subjects, 586 MS patients (91.6%) and 17 HCs (5.4%) resulted CCSVI-positive ($p<0.0001$), with an OR of 190.23 (95% CI 108.37-333.90) for patients with MS compared to HCs. The prevalence of five Zamboni criteria was higher in the MS group than in the HC group

**Table 3:** Prevalence of Zamboni Criteria and CCSVI by Disease Group (MS, HCs).

| | MS patients (n=640) | HCs (n=315) | OR (95% CI) | *p* |
|---|---|---|---|---|
| **CCSVI presence** | 586 (91.6%) | 17 (5.4%) | 190.23 (108.37-333.90) | <0.0001 |
| **Criterion 1** | 289 (45.2%) | 25 (7.9%) | 9.55 (6.17-14.79) | <0.0001 |
| **Criterion 2** | 585 (91.4%) | 78 (24.8%) | 32.32 (22.18-47.10) | <0.0001 |
| **Criterion 3** | 573 (89.5%) | 52 (16.5%) | 43.26 (29.27-63.92) | <0.0001 |
| **Criterion 4** | 221 (34.5%) | 0 | 333.17 (20.69-5364.14) | <0.0001 |
| **Criterion 5** | 82 (12.8%) | 0 | 93.21 (5.76-1507.97) | 0.0014 |

CCSVI=chronic cerebrospinal venous insufficiency; HCs=healthy controls; MS=multiple sclerosis; OR=odds ratio.

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-123

**Table 4:** Correlation of CCSVI with Clinical Subtype, Gender, Age and Disease Duration (n=640).

| Variable | CCSVI prevalence | *p* |
|---|---|---|
| **Clinical subtypes** | | |
| - CIS | 9 (90.0%) | |
| - RRMS | 377 (90.6%) | 0.632 |
| - SPMS | 144 (92.7%) | |
| - PPMS | 56 (94.9%) | |
| **Gender** | | |
| - male | 238/248 (96.0%) | 0.002 |
| - female | 348/392 (88.8%) | |
| **Age** | | |
| <45 years | 308/345 (89.3%) | 0.006 |
| >=45 years | 278/295 (94.2%) | |
| **Disease duration** | | |
| <5 years | 156/176 (88.6%) | |
| 5-9 years | 152/164 (92.7%) | 0.429 |
| 10-14 years | 130/141 (92.2%) | |
| >14 years | 148/159 (93.1%) | |

CCSVI=chronic cerebrospinal venous insufficiency; CIS=clinically isolated syndrome; PPMS=primary progressive multiple sclerosis; RRMS=relapsing remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis.

**Table 5:** Impact of the New echo-colour Doppler Criteria.

| | Old criteria (n=477) | New criteria (n=163) | *p* |
|---|---|---|---|
| **CCSVI presence** | 450 (94.3%) | 136 (83.4%) | <0.001 |
| **CCSVI presence (without criterion 2)** | 334 (70.0%) | 96 (58.9%) | 0.012 |
| **CCSVI by clinical subtype** | | | |
| - CIS | 0/1 | 9/9 | - |
| - RRMS | 279/294 (94.9%) | 98/122 (80.3%) | <0.001 |
| - SPMS | 125/133 (94.0%) | 19/22 (86.4%) | 0.400 |
| - PPMS | 46/49 (93.9%) | 10/10 (100%) | 0.989 |
| **Positive by criterion** | | | |
| - 1 | 230 (48.2%) | 59 (36.2%) | 0.010 |
| - 2 | 455 (95.4%) | 130 (79.8%) | <0.001 |
| - 3 | 433 (90.8%) | 140 (85.9%) | 0.107 |
| - 4 | 168 (35.2%) | 53 (32.5%) | 0.595 |
| - 5 | 64 (13.4%) | 18 (11.0%) | 0.517 |

CCSVI=chronic cerebrospinal venous insufficiency; CIS=clinically isolated syndrome; PPMS=primary progressive multiple sclerosis; RRMS=relapsing remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis.

($p$<0.001) with statistically significant ORs for each of the five criteria (Table 3).

Moreover, Table 4 shows correlations of CCSVI with clinical subtypes, gender, age and disease duration. CCSVI prevalence was equal in the different clinical subtypes ($p$=0.632), and no differences in CCSVI prevalence were found according to disease duration ($p$=0.429). By contrast, the presence of CCSVI showed asignificant correlation with gender ($p$=0.002) and age group ($p$=0.006), with higher CCSVI prevalence observed in males and older patients. We also found a positive correlation between the VHISS score and age (r=0.254, $p$<0.001), disease duration (r=0.201, $p$<0.001) and EDSS (r=0.287, $p$<0.001).

The prevalence rate of CCSVI in MS patients examined between 2010 and 2014 was compared before and after the November 2011 criteria revision, i.e., by the old and new ECD criteria (Table 5). The overall CCSVI prevalence rate changed from 94.3% with the old criteria to 83.4% with the new ones ($p$<0.001), and from 70% to 58.9% without considering criterion

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-123

**Table 6:** Distribution of Internal Jugular Vein Defective Valves (Movement Alterations) with B-Mode and M-Mode Analysis.

| | B-Mode | | | | M-Mode | | |
|---|---|---|---|---|---|---|---|
| **Valve types** | 280 | | | Evaluable valves (n=184) | 280 | | |
| | Right side (n/%) | Left side (n/%) | Total (n/%) | % | Right side (n/%) | Left side (n/%) | Total (n/%) |
| **- Mobile** | 63/22.55 | 27/9.6 | 90/32.1 | 48.9 | 58/20.7 | 35/12.5 | 93/33.2 |
| **- Slightly mobile** | 25/8.9 | 50/17.9 | 75/26.8 | 40.8 | 80/28.6 | 59/21.1 | 139/49.7 |
| **- Not mobile** | 3/1.1 | 16/5.7 | 19/6.8 | 10.3 | 2/0.7 | 4.6/16.4 | 48/17.1 |
| **- Not evaluable** | 49/17.5 | 47/16.8 | 96/34.3 | | 0/0 | 0/0 | 0/0 |

2 (non-mandatory after ECD revision). The impact of the new ECD criteria was significant for the CCSVI diagnosis in Relapsing remitting multiple sclerosis (RRMS) patients: the prevalence rate changed from 94.9 to 80.3% ($p<0.001$). The prevalence of two positive criteria (criterion 1 and criterion 2) diminished significantly after the November 2011 ECD revision: criterion 1 positivity changed from 48.2% to 36.2% ($p=0.010$) before/after the revision, criterion 2 from 95.4% to 79.8% ($p<0.001$). A similar trend was observed for positivity of the third criterion (4.9% absolute reduction, $p=0.107$),whereas the fourth and fifth criteria did not change significantly ($p=0.595$; $p=0.517$). Table 6 shows the distribution of valvular defects (movement alterations) in subjects analyzed with the revised Protocol (B-mode *vs*. M-mode analysis). B-mode analysis resulted in 96 (34.3%) not evaluable valvular defects and, on the total number of evaluable valves (n=184), in 91 true positive, 72 true negative, 18 false negative and 3 false positive cases. The overall B-mode diagnostic accuracy was 88.6% (95% CI: 84.0%-93.2%), with a sensitivity of 83.5% (95% CI: 75.2%-89.9%), a specificity of 96.0% (95% CI: 88.8%-99.2%), a positive predictive value of 96.8% (95% CI: 91.0%-99.3%) and a negative predictive value of 80.0% (95% CI: 70.3%-87.7%).

In MS patients, the strength of inter-observer agreement changed from moderate (k=0.532, 95% CI 0.404-0.660) to good (k=0.761, 95%CI 0.639-0.884) before/after the revision. Finally, this agreement would have changed from good (k=0.628, 95% CI 0.536-0.720) with the intracranial criterion to excellent (k=0.830, 95% CI 0.786-0.874) without it.

## DISCUSSION

Since the first publication of Zamboni, the debate about the real meaning of the new nosological entity known as CCSVI and its relationship with MS has found researchers on diametrically opposing positions. In fact, the prevalence of CCSVI highlighted in case-control studies has ranged from 0% to 100% in MS patients and from 0% to 36% in controls.[1,3,11,12,26,29,41] Non-controlled studies have shown a higher prevalence. This fact sharply contrasts with the finding of significant intraluminal (septa, webs, membranes, fixed and rudimental valves, or wall stenosis) or valvular (tricuspid valves, enlarged and malposition valve leaflets, small accessory valve leaflets) abnormalities during post-mortem examinations or surgical procedures.[42-44] These defective valves reduce the normal venous outflow from the brain as proven with different methodologies for flow assessment.[45-47] Beyond the deeply held convictions of individual researchers, the heterogeneity of Doppler studies can be reduced to three variables: the participant observed, the measuring instrument and procedure, and the observer.[36] Because this is a case-control study, we were not able to select the participants about certain variables such as age, clinical form and disability. However, we checked the possible sources of variability related to the procedure (controlled breathing, head and body position, degree of hydration, time of day of the examination, cervical muscle relaxation), adapting it to the investigative protocols used both before and after November 2011.[48,49] Moreover, we also used the same ECD unit equipped with software upgraded in November 2011. As reported in many studies, ECD is a highly observer-dependent examination.[12,15,50] Therefore, inter-operator variability is a major source of heterogeneity.[11,12,41] Because experience in venous diagnostic ultrasonography and appropriate training in Zamboni's courses appear to reduce inter-operator variability, the present study utilised two vascular sonographers experienced with venous diseases who had undergone the same special training.[12,15,41,50] Another important aspect is that it seems to be very difficult for an observer to operate in a blind fashion due to the easy detection of subjects with a high degree of disability (severe walking disability needing evident assistance).[36,41] That is why at first all the evaluations were conducted by each investigator as open trial. At a later stage of the study all exams recorded by one operator were subjected to a blind evaluation from the other researcher. After this review, we obtained 92.4% agreement for MS patients and 96.3% for HCs between the two examiners in the assessments. In our study, the overall prevalence of CCSVI in MS patients in the observation period (2010-2014) was 91.6%, a high prevalence when compared to HCs (5.4%). This result takes more importance considering that it is relative to the case-control study with the highest number of subjects compared to similar researches so far published in the literature. As reported by Lanzillo, we also found a significant correlation between the prevalence of CCSVI and age ($p=0.006$) and no correlation with regard to MS clinical forms ($p=0.632$) or disease duration ($p=0.429$).[51] In contrast, we found a positive correlation with gender (male=96.0%, female=88.8%, $p=0.002$). Another interesting finding of our study is related to the meaning of the VHISS score. As we know, it is an ordinal measure of the overall extent and number of CCSVI criteria,

with higher VHISS values indicating flow pattern anomalies of greater severity.[10] In our experience, we found a positive correlation between the VHISS score and age (r=0.254, $p<0.001$), disease duration (r=0.201, $p<0.001$) and EDSS (r=0.287, $p<0.001$). The data analysis before and after the introduction of the new ECD criteria revealed a significant reduction of the prevalence of CCSVI (from 94.3% to 83.4%, $p<0.001$) (Table 5). As this result is not due to a different distribution of MS clinical forms between the two evaluation periods, it can only be attributed to the revised recommendations for the investigation and screening of CCSVI.[10] As shown in Table 5, positivity on the first criterion was significantly reduced due to the revision ($p=0.010$). We observed a similar trend, although not significant ($p=0.107$), for third criterion positivity (4.9% reduction); whereas the fourth and fifth criteria did not change significantly ($p=0.595$; $p=0.517$). As also reported by other authors[16,52] we consider fundamental the M-mode analysis for its ability to demonstrate motility, competence or any valvular abnormalities frequently not detectable with the B-mode analysis, in fact its sensitivity respect to M-mode was 83.5% (95% CI: 75.2%-89.9%) with 18 false negative cases. The rate of non-evaluable valves with the B-mode analysis (34.3%) decreased to 0% by using the M-Mode analysis. This reduction seems mainly due to M-mode ability in detecting not mobile (17.1%) or slightly mobile (49.7%) valves compared to B-mode (respectively 10.3% and 40.8%). Similarly, our study clearly demonstrates the importance of the second criterion in estimating the CCSVI prevalence. After the revision, indeed, the prevalence with and without this criterion has changed from 83.4% to 58.9%. Also before the revision by removing this criterion, the prevalence decreases from 94.3% to 70%. The significant reduction observed after the revision in positivity on the second criterion ($p<0.001$), could likely be explained by the observation of less serious clinical and haemodynamic cases (RR forms [+13.2%], SP forms [-14.4%], PP forms [-4.2%]) after the review. Our data showed a higher prevalence of CCSVI in MS patients compared to HCs, increasing with age and being more frequent in males. By contrast, no correlation was found regarding MS clinical forms or disease duration. In MS patients with CCSVI we also detected a positive correlation between impaired cerebral outflow (identified by the VHISS score) and age, disease duration and EDSS.

## CONCLUSION

Vascular survey protocols must reduce their inter-operator variability, and only specific training of vascular sonographers with high competence in venous ultrasonography can achieve this goal. Achieving reproducibility in investigations relative to patients and methods seems to be easier when applying the relevant protocols. In fact, the use in our study of the new ECD criteria introduced in 2011 made the method of investigation more stringent, reducing the prevalence of CCSVI in MS patients compared with examinations using the old criteria (83.4% *vs*. 94.3%). This result would seem to be due to changes introduced in the first and third criteria. Our study proves that criterion 2 is a disagreement element for its difficult reproducibility, even between experienced and trained operators. Indeed, the inter-observer agreement has changed from good to excellent without this criterion. Nevertheless, considering this criterion important for a better assessment of cerebral hemodynamic this problem could be overcome with use of MRI-ultrasound fusion techniques[53] only in selected cases (post-surgical monitoring, patients with positivity of a single criterion evaluated by the revised protocol for CCSVI screening).

## ACKNOWLEDGEMENTS

Our personal gratitude for the support provided to this research goes to the Italian Rotary Club – 2040 District – Rho (MI), and particularly to a special friend Della Vedova MR, M.D., for all that she has given in these years of friendship and for her active collaboration in data collection of this study.

"The unexamined life is not worth living for a human being": sincere thanks to our friend Franz Schelling for his valuable suggestions in writing this work.

## AUTHOR'S CONTRIBUTION

MF was involved in the study concept/study design, data acquisition, data analysis/interpretation, manuscript drafting, literature research, and clinical studies. EN performed the statistical analysis and data analysis/interpretation, drafted the manuscript, and performed the literature research. RP was involved in the data acquisition and clinical studies.

## CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

## REFERENCES

1. Zamboni P, Galeotti R, Melegatti E, et al. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2009; 80(4): 392-399. doi: 10.1136/jnnp.2008.157164

2. Khan O, Filippi M, Freedman MS, et al. Chronic cerebrospinal venous insufficiency and multiple sclerosis. *Annals Neur*. 2010; 67(3): 286-290. doi: 10.1002/ana.22001

3. Al-Omari MH, Rousan LA. Internal jugular vein morphology and hemodynamics in patients with multiple sclerosis. *Int Angiol*. 2010; 29(2): 115-120. Web site. http://search.proquest.com/openview/0f879454d0bc2e8bf83897fdffa952a7/1?pq-origsite=gscholar&cbl=46530. Accessed December 21, 2016.

4. Farina M, Novelli E, Pagani R. Cross-sectional area variations of internal jugular veins during supine head rotation in multiple sclerosis patients with chronic cerebrospinal venous insufficiency: A prospective diagnostic controlled study with duplex ultrasound investigation. *BMC Neurol*. 2013; 13: 162.

doi: 10.1186/1471-2377-13-162

5. Marchione P, Morreale M, Giacomini P, et al. Ultrasonographic evaluation of cerebral arterial and venous haemodynamics in multiple sclerosis: A case-control study. *PLoS One*. 2014; 9(10): e111486. doi: 10.1371/journal.pone.0111486

6. Reekers JA, Lee MJ, Belli AM, et al. Cardiovascular and interventional radiology society of Europe commentary on the treatment of chronic cerebrospinal venous insufficiency. *Cardiovasc Intervent Radiol*. 2011; 34(1): 1-2. doi: 10.1007/s00270-010-0050-5

7. Vedantham S, Benenati JF, Kundu S, et al. Interventional endovascular management of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis: A position statement by the Society of Interventional Radiology, endorsed by the Canadian Interventional Radiology Association. *J Vasc Interv Radiol*. 2010; 21(9): 1335-1337. doi: 10.1016/j.jvir.2010.07.004

8. Wattjes MP, van Oosten BW, de Graaf WL, et al. No association of abnormal cranial venous drainage with multiple sclerosis: A magnetic resonance venography and flow-quantification study. *J Neurol Neurosurg Psychiatry*. 2011; 82(4): 429-435. doi: 10.1136/jnnp.2010.223479

9. Zivadinov R, Poloni GU, Marr K, et al. Decreased brain venous vasculature visibility on susceptibility-weighted imaging venography in patients with multiple sclerosis is related to chronic cerebrospinal venous insufficiency. *BMC Neurol*. 2011; 11: 128. doi: 10.1186/1471-2377-11-128

10. Nicolaides AN, Morovic S, Menegatti E, Viselner G, Zamboni P. Screening for chronic cerebrospinal venous insufficiency (CCSVI) using ultrasound: Recommendations for a protocol. *Func Neurol*. 2011; 26(4): 229-248. Web site. http://search.proquest.com/openview/5a57f945fc9a02ae19342407758b1fe1/1?pq-origsite=gscholar&cbl=34390. Accessed December 21, 2016.

11. Zwischenberger A, Beasley MM, Davenport DL, Xenos ES. Meta-analysis of the correlation between chronic cerebrospinal venous insufficiency and Multiple Sclerosis. *Vasc Endovascul Surg*. 2013; 47(8): 620-624. doi: 10.1177/1538574413503562

12. Tsivgoulis G, Sergentanis TN, Chan A, et al. Chronic cerebrospinal venous insufficiency and multiple sclerosis: A comprehensive meta-analysis of case-control studies. *Ther Adv Neurol Disord*. 2014; 7(2): 114-136. doi: 10.1177/1756285613499425

13. Tsivgoulis G, Faissner S, Voumvourakis K, et al. "Liberation treatment" for chronic cerebrospinal venous insufficiency in multiple sclerosis: The truth will set you free. *Brain Behav*. 2015; 5(1): 3-12. doi: 10.1002/brb3.297

14. Jedynak W, Cieszanowski A. Is there any relation between chronic cerebrospinal venous insufficiency and multiple sclerosis? - A critical review. *Pol J Radiol*. 2014;79: 131-136. doi: 10.12659/PJR.890379

15. Menegatti E, Genova V, Tessari M, et al. The reproducibility of colour Doppler in chronic cerebrospinal venous insufficiency associated with multiple sclerosis. *Int Angiol*. 2010; 29(2): 121-126. Web site. http://www.minervamedica.it/en/journals/international-angiology/article.php?cod=R34Y2010N02A0121. Accessed December 21, 2016.

16. Tromba L, Blasi S, Vestri A, Kiltzanidi D, Tartaglia F, Redler A. Prevalence of chronic cerebrospinal venous insufficiency in multiple sclerosis: A blinded sonographic evaluation. *Phlebology*. 2015; 30(1): 52-60. doi: 10.1177/0268355513512823

17. Chambers B, Chambers J, Cameron H, et al. Chronic cerebrospinal venous insufficiency is not more prevalent in patients with mild multiple sclerosis: A sonographer-blinded, case-control ultrasound study. *Mult Scler*. 2013; 19(6): 749-756. Web site. http://journals.sagepub.com/doi/abs/10.1177/1352458512459986. Accessed December 21, 2016.

18. Weinstock-Guttman B, Ramanathan M, Marr K, et al. Clinical correlates of chronic cerebrospinal venous insufficiency in multiple sclerosis. *BMC Neurol*. 2012; 12: 26. doi: 10.1186/1471-2377-12-26

19. Ciccone MM, Galeandro AI, Scicchitano P, et al. Multigate quality Doppler profiles and morphological/hemodynamic alterations in multiple sclerosis patients. *Curr Neurovasc Res*. 2012; 9(2): 120-127. doi: 10.2174/156720212800410849

20. Patti F, Nicoletti A, Leone C, et al. Multiple Sclerosis and CCSVI: A population-based case control study. *PLoS One*. 2012; 7(8): e41227. doi: 10.1371/journal.pone.0041227

21. Lanzillo R, Mancini M, Liuzzi R, et al. Chronic cerebrospinal venous insufficiency in multiple sclerosis: A highly prevalent age-dependent phenomenon. *BMC Neurol*. 2013; 13(20): 20. doi: 10.1186/1471-2377-13-20

22. Zaniewski M, Kostecki J, Kuczmik W, et al. Neck duplex Doppler ultrasound evaluation for assessing chronic cerebrospinal venous insufficiency in multiple sclerosis patients. *Phlebology*. 2013; 28(1): 24-31. doi: 10.1258/phleb.2011.011070

23. Imperiale D, Melis F, Giaccone C, et al. Chronic cerebrospinal venous insufficiency in multiple sclerosis: A sonographer-blinded case-control study. *Clin Neurol Neurosurg*. 2013; 115(8): 1394-1398. doi: 10.1016/j.clineuro.2013.01.003

24. Krsmanović Ž, Živković M, Lepić T, et al. Small internal jugular veins with restricted outflow are associated with severe

multiple sclerosis: A sonographer-blinded, case-control ultrasound study. *BMC Neurol*. 2013; 13: 90. doi: 10.1186/1471-2377-13-90

25. Kim ES, Diaconu C, Baus L, et al. Chronic cerebrospinal venous insufficiency: Pitfalls and perils of sonographic assessment. *J Ultrasound Med*. 2015; 34(6): 1097-1106. doi: 10.7863/ultra.34.6.1097

26. Blinkenberg M, Akeson P, Sillesen H, et al. Chronic cerebrospinal venous insufficiency and venous stenoses in multiple sclerosis. *Acta Neurol Scand*. 2012; 126(6): 421-427. doi: 10.1136/jnnp.2008.157164

27. Leone MA, Raymkulova O, Naldi P, et al. Chronic cerebrospinal venous insufficiency is not associated with multiple sclerosis and its severity: A blind-verified study. *PLoS One*. 2013; 8(2): e56031. doi: 10.1371/journal.pone.0056031

28. Barreto AD, Brod SA, Bui T-T, et al. Chronic cerebrospinal venous insufficiency: Case–control neurosonography results. *Ann Neurol*. 2013; 73(6): 721-728. doi: 10.1002/ana.23839

29. Comi G, Battaglia MA, Bertolotto A, et al. Observational case-control study of the prevalence of chronic cerebrospinal venous insufficiency in multiple sclerosis: Results from the CoSMo study. *Mult Scler*. 2013; 19(11): 1508-1517. doi: 10.1177/1352458513501231

30. Rodger IW, Dilar D, Dwyer J, et al. Evidence against the involvement of chronic cerebrospinal venous abnormalities in multiple sclerosis. A case-control study. *PLoS One*. 2013; 8(8): e72495. doi: 10.1371/journal.pone.0072495

31. Laughlin S, Macgowan CK, Traubici J, et al. No evidence for impairment of venous hemodynamics in children or young adults with pediatric-onset multiple sclerosis. *AJNR Am J Neuroradiol*. 2013; 34(12): 2366-2372. doi: 10.3174/ajnr.A3661

32. Costello F, Modi J, Lautner D, et al. Validity of the diagnostic criteria for chronic cerebrospinal venous insufficiency and association with multiple sclerosis. *CMAJ*. 2014; 186(11): E418-E426. doi: 10.1503/cmaj.131431

33. Leone MA, Raymkulova O, Lochner P, et al. Chronic cerebrospinal venous insufficiency is not associated with chronic venous disorders: A case-control study. *Phlebology*. 2015; 30(10): 736-738. doi: 10.1177/0268355514544782

34. Baracchini C, Valdueza JM, Del Sette M, et al. CCSVI and MS: A statement from the European Society of neurosonology and cerebral hemodynamics. *J Neurol*. 2012;259(12): 2585-2589. doi: 10.1007/s00415-012-6541-3

35. Laukontaus SJ, Kagayama T, Lepäntalo M, et al. Doppler ultrasound examination of multiple sclerosis patients and control participants: Inter-observer agreement and association with disease. *Eur J Vasc Endovasc Surg*. 2013; 46(4): 466-472. doi: 10.1016/j.ejvs.2013.07.003

36. Leone MA, Raymkulova O, Lucenti A, et al. A reliability study of colour-Doppler sonography for the diagnosis of chronic cerebrospinal venous insufficiency shows low inter-rate agreement. *BMJ Open*. 2013; 3(11): e003508. doi: 10.1136/bmjopen–2013–003508

37. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol*. 2011; 69(2): 292-202. doi: 10.1002/ana.22366

38. Feldman LS, Anidjar M, Metrakos P, et al. Optimization of cardiac preload during laparoscopic donor nephrectomy: A preliminary study of central venous pressure versus esophageal Doppler monitoring. *Surg Endosc*. 2004; 18(3): 412-416. doi: 10.1007/s00464-003-8907-1

39. Mathew JP, Newman MF. *Hemodynamic and Related Monitoring*. 2nd ed. Philadelphia, USA: Lippincott William and Wilkins; 2005.

40. Stolz E, Kaps M, Kern A, et al. Transcranial color-coded duplex sonography of intracranial veins and sinuses in adults: Reference data from 130 volunteers. *Stroke*. 1999; 30(5): 1070-1075. doi: 10.1161/01.STR.30.5.1070

41. Bastianello S, Romani A, Viselner G, et al. Chronic cerebrospinal venous insufficiency in multiple sclerosis: Clinical correlates from a multicenter study. *BMC Neurol*. 2011; 26(11): 132. doi: 10.1186/1471-2377-11-132

42. Diaconu CI, Staugaitis SM, Fox RJ, Rae-Grant A, Schwanger C, McBride JM. A technical approach to dissecting and assessing cadaveric veins pertinent to chronic cerebrospinal venous insufficiency in multiple sclerosis. *Neurol Res*. 2012; 34(8): 810-818. doi: 10.1179/1743132812Y.0000000071

43. Diaconu CI, Staugaitis S, McBride J, et al. A Pathologic evaluation of chronic cerebrospinal venous insufficiency (CCSVI). *Neurology*. 2012; 78(1): 5-15. Web site. http://www.neurology.org/content/78/1_Supplement/P05.125.short. Accessed December 21, 2016.

44. Pedriali M, Zamboni P. The pathology of the internal jugular vein wall in multiple sclerosis. *J Mult Scler*. 2015; 2: 160. doi: 10.4172/2376-0389.1000160

45. Zamboni P, Menegatti E, Conforti P, Shepherd S, Tessari M, Beggs C. Assessment of cerebral venous return by a novel plethysmography method. *J Vasc Surg*. 2012; 56(3): 677-685.e1. doi: 10.1016/j.jvs.2012.01.074

46. Beggs C, Shepherd S, Zamboni P. Cerebral venous outflow

resistance and interpretation of cervical plethysmography data with respect to the diagnosis of chronic cerebrospinal venous insufficiency. *Phlebology*. 2014; 29(3): 191-99. doi: 10.1258/phleb.2012.012039

47. Mancini M, Lanzillo R, Liuzzi R, et al. Internal jugular vein blood flow in multiple sclerosis patients and matched controls. *PLoS One*. 2014; 9(3): e92730. doi: 10.1371/journal.pone.0092730

48. Lobato EB, Florete OG, Paige GB, Morey TE. Cross-sectional area and intravascular pressure of the right internal jugular vein during anesthesia: Effects of Trendelenburg position, positive intrathoracic pressure, and hepatic compression. *J Clin Anesth*. 1998; 10(1): 1-5. doi: 10.1016/S0952-8180(97)00189-X

49. Valdueza JM, von Munster T, Hoffman O, Schreiber S, Einhäupl KM. Postural dependency of the cerebral venous outflow. *Lancet*. 2000; 355(9199): 200-201. doi: 10.1016/S0140-6736(99)04804-7

50. Zivadinov R, Marr K, Cutter G, et al. Prevalence, sensitivity, and specificity of chronic cerebrospinal venous insufficiency in MS. *Neurology*. 2011; 77(2): 138-144. doi: 10.1212/WNL.0b013e318212a901

51. Lanzillo R, Mancini M, Liuzzi R, et al. Chronic cerebrospinal venous insufficiency in multiple sclerosis: A highly prevalent age-dependent phenomenon. *BMC Neurol*. 2013; 13(20): 13-20. doi: 10.1186/1471-2377-13-20

52. Menegatti E, Tessari M, Gianesini S, et al. Human internal jugular valve M-mode ultrasound characterization. *Curr Neurovasc Res*. 2014; 11(2): 149-155. doi: 10.2174/1567202611666140408094014

53. Schreiber SJ, Valdueza JM, Doepp F. Transcranial color coded sonography: Advanced approach using ultrasound fusion imaging. *IJCNMH*. 2014; 1(Suppl 1): S16. doi: 10.21035/ijcnmh.2014.1(Suppl.1).S16

NEURO

Openventio PUBLISHERS

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-124

Short Communication

*Corresponding author
**Avinash Kumar Kanodia**, MD, DM, FRCR
Department of Radiology
Ninewells Hospital
Dundee, UK
**E-mail:** avinash.kanodia@nhs.net

*Volume 4 : Issue 1*
*Article Ref. #: 1000NOJ4124*

*Article History*
**Received:** March 26th, 2017
**Accepted:** May 5th, 2017
**Published:** May 8th, 2017

*Citation*
Abdelsadg M, Kanodia AK, Badran K, Abdelsadig K, Mowle D. Management of CSF leak following incidental durotomy during lumbar spinal surgery: Is flat bed necessary? *Neuro Open J*. 2017; 4(1): 11-15. doi: 10.17140/NOJ-4-124



# Management of CSF Leak Following Incidental Durotomy during Lumbar Spinal Surgery: Is Flat Bed Necessary?

**Mohamed Abdelsadg, MBBS, PgDip[1]; Avinash Kumar Kanodia, MD, DM, FRCR[2*]; Khaled Badran, MBBS[1]; Khalid Abdelsadig, MBBS[3]; David Mowle, FRCS[1]**

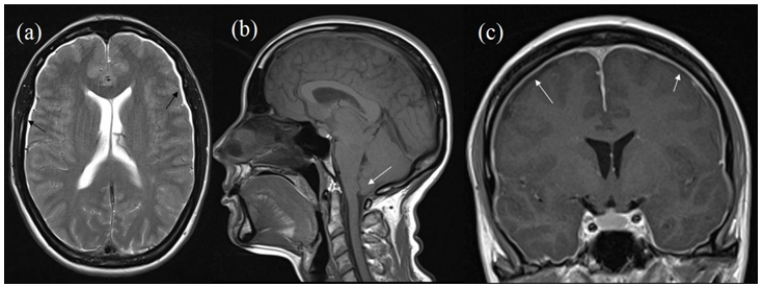
*[1]Department of Neurosurgery, Ninewells Hospital, Dundee, UK*
*[2]Department of Radiology, Ninewells Hospital, Dundee, UK*
*[3]Department of Accident and Emergency, North Manchester General Hospital, Manchester, UK*

Incidental durotomy (ID) is a term used to describe unintentional opening of the dura mater during spinal surgery. Although, commonly referred to as dural tear; most recent scholars are circumventing the use of the latter term, as it could imply an element of carelessness when none were necessarily present. Therefore, ID, unintended durotomy, unintentional durotomy or simply just dural opening, have been recommended to replace the term dural tear.[1,2]

The reported incidence of ID of spinal surgeries ranges from 0.1-13.7%. Despite being one of the most common complications of spinal surgery, it remains often underreported because of the lack of morbidity in the most of cases.[3] ID is often associated with cerebrospinal fluid (CSF) leakage from the subarachnoid space through the dural defect.[1]

CSF leakage can lead to several complications resulting from loss of CSF volume (decreasing the brain's supportive cushion), which can lead to symptoms of intracranial hypotension (presenting with nausea, vomiting, postural headache, tinnitus, vertigo, etc) (Figure 1). It can also lead to more serious yet less common complications such as CSF fistula formations, and meningitis.[4] Since some of the aforementioned symptoms are improved in the recumbent position, patients are often advised to remain flat in bed for a period of time (ranging from 1-10 days) after the spine surgery.[4] Previous studies showed that bed rest without intervention is ineffective in treating an accidental durotomy.[5] However, following dural repair the need for flat bed rest is still an area of ongoing debate.[6]

**Figure 1:** Low Intracranial Pressure Following CSF Leak. T2 Axial (a) Shows Thin Subdural Fluid Collection (Black Arrows). (b) T1 Sagittal Shows Sagging of Brainstem and Cerebellar tonsillar Descent (White Arrow). (c) T1 Coronal Post-Contrast Shows Diffuse Pachymeningeal Enhancement (White Arrows).




Currently, there is no concrete evidence to support the beneficial effects of flat bed rest following ID nor there a consensus about the period of this bed rest. Therefore, post-operative instructions for patients with ID remain controversial and rely heavily on the previous experience/preference of the treating surgeon(s).[5]

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-124

This debatable topic was first addressed by Hodges et al[6] who conducted a retrospective review of 20 patients with ID and resultant CSF leakage treated without mandatory bed rest following the dural repair. In 19 cases the CSF leakage was detected and repaired (with non-absorbable stiches and fibrin glue) during the initial procedure, and in 1 patient the defect was repaired 2 weeks after the surgery. All 20 patients had their symptoms monitored for 1 week, and delayed follow-up at a minimum of 10 months took place. During a week from surgery only 2 patients (10%) reported headache, 2 patients (10%) reported nausea, and 1 patient (5%) reported tinnitus.[6] Worth mentioning; similar symptoms were reported in patients treated with bed rest following ID and CSF leak.[1] The study concluded that 75% of the patients had no symptoms related to the ID despite being mobilised immediately post-operative.[6]

In 2013 Low et al[7] addressed the same topic, through a retrospective study. They looked at a year data of a single centre for all patients who incurred an accidental durotomy and were repaired intraoperatively.[7] Their notes were reviewed for evidence of complications for a minimum of 12 months after surgery. Out of 889 patients who had lumbar surgery 61 patients (6.8%) had ID and CSF leak. Twenty six patients were mobilised immediately following surgery, 9 patients were mobilised after 48 hours and 26 patients were prescribed flat bed rest for 72 hours or more. The review concluded that there was no statistical significance between the day of mobilisation and the rate of complication. Similar results were reproduced by other study promoting early mobilisation.[8]

Bonanos et al[9] prospectively audited flat bed rest for managing ID in lumbar spine surgery over a period of 6 months. The study showed that flat bed rest was used in 20 patients with ID for an average of 4 days ranged from 1-10 days. They concluded that flat bed rest does not reduce morbidity following ID, and in fact was deemed unnecessary.[9]

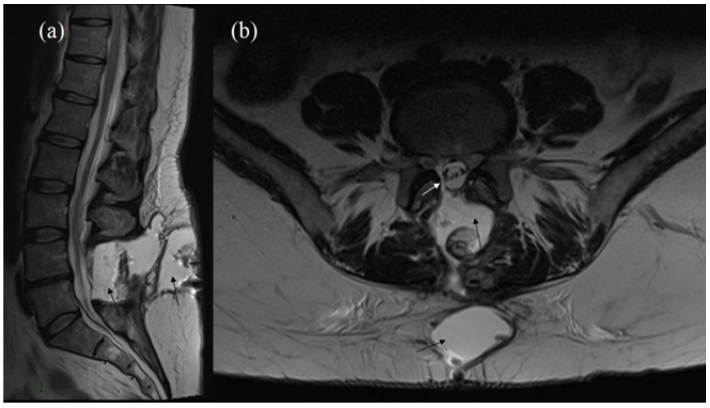
A German national survey looking at the intra- and post-operative management of accidental durotomy in lumbar spine surgery, showed no consensus concerning the management. The survey however, concluded that despite not being proved to reduce the rate of cerebrospinal fluid fistulas, bed rest is still frequently used. While period of flat bed rest prolongs the hospital stay with additional costs and potentially higher rate of medical complications, the survey warranted a multicentre trial to address the topic.[10] Another survey looking at 3 nations (German, Swiss, and Austrian) practice regarding the management of ID, revealed once more the substantial heterogeneity in the management. Nonetheless; there is a trend towards early mobilisation if the ID has been closed sufficiently with no participant favouring bed rest for more than 72 hours.[11]

One study compared rates of complications between patients who undergo flat bed rest (for over 24 hours) following ID to those allowed to mobilize early (within the first 24 hours post-operative ).[12] The study concluded that there was no statistically significant difference between both groups in regards to post-durotomy related neurological complications, wound complications and need for revision surgery. However, there was a statistically significant decrease in the incidence of total medical complications in the early mobilized group (0% *vs.* 50%, $p$=0.0003).[12]

In our own practice, we have not done a full fledged study on the management of CSF leak following ID. However, Intra-operatively and more often on imaging, it is possible to clearly localise the site of intradural communication. We have practised open repair, lumbar drain insertion and lately injection of dural sealing agents with very good results. Prescription of flat bed rest remains controversial in our local practice. Nonetheless the senior author encourages early mobilisation for all his patients following satisfactory repair of the durotomy; with increasingly encouraging results for early mobilisation, other consultants in the department have started adopting early mobilisation approach for their patients.

Some examples from our own practice are included here. Figure 2 shows a patient who was treated with lumbar drain

**Figure 2:** CSF Leak Following ID. T2 Sagittal (a) and Axial (b) Images Showing a Prominent CSF/Fluid Collection (Black Arrows) Following ID. White Arrow in (b) Shows the Site of Dural Communication, Leading to the Fluid Collection. Patient has been Treated with Lumbar Drain and Flat Bed Rest with Good Outcome.



Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-124

and flat bed rest, following which the patient improved and returned to normal. Figure 3 shows a patient with ID, treated with open repair and flat bed rest, with good outcome. Even prominent collections managed with dural sealing agent; have been successfully treated without flat bed rest (Figure 4). However, we do come across occasional patients who have had unfavourable outcome with persistent symptoms despite open repair and bed rest, we have successfully treated such patients with glue injection, it is often important to localise the site of communication in such patients (Figure 5).

## CONCLUSION

Despite the fact that a period of flat bed rest following ID and CSF leak is commonly advised, it is by no means accepted as a standard practice. Although, there is a clear need for first class evidence to address the topic; yet, the current available evidence supports early mobilisation following sufficient dural repair. We understand the difficulties in running such trial as standardizing the size and site of the durotomy, the underlying spinal pathology, patients' comorbidities/healing potentials, and closure tech-

**Figure 3:** CSF Leak Following ID. T2 Sagittal (a) and Axial (b) Images Showing a Prominent CSF/Fluid Collection (Black Arrows) Following ID. The Site of Communication Not Clearly Identified. Patient was Treated with Open Repair and Flat Bed Rest with Good Outcome.

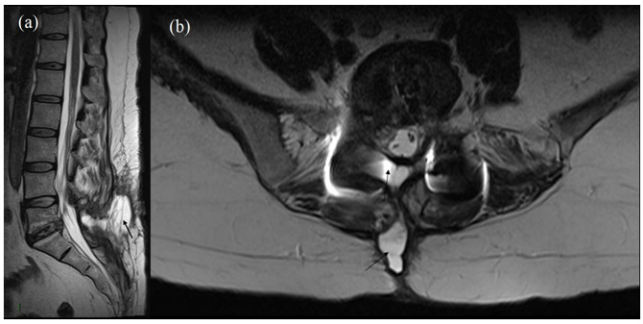
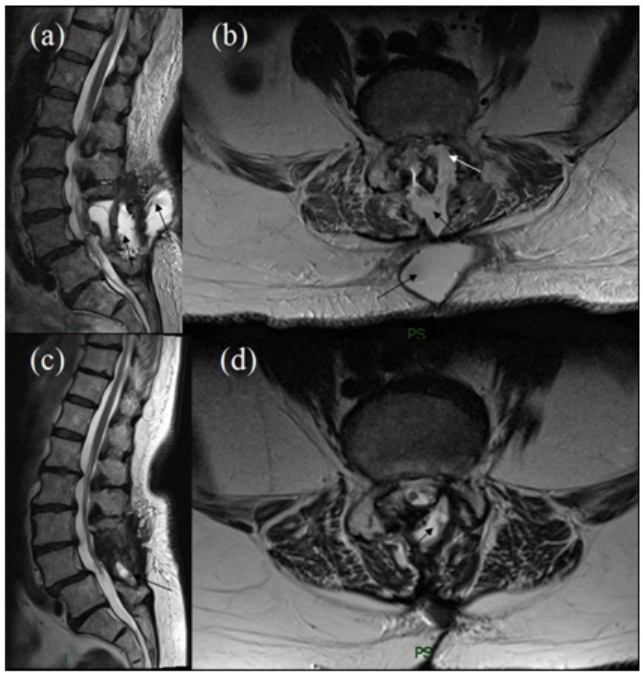


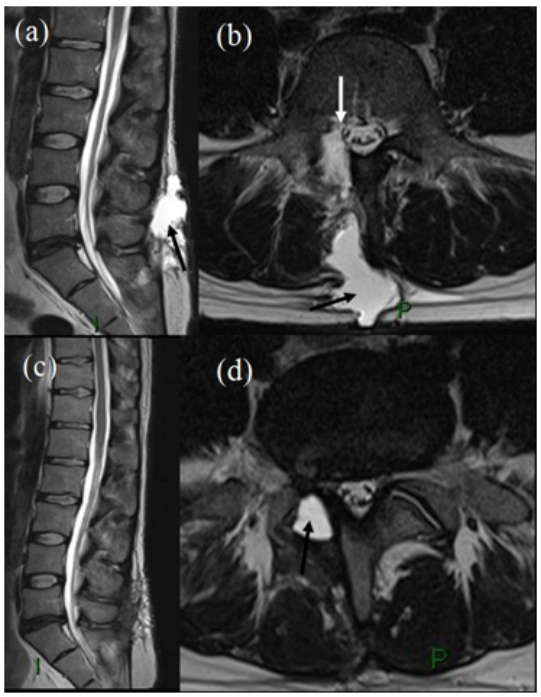

**Figure 4:** CSF Leak Following ID. T2 Sagittal (a) and Axial (b) Images (Above) Showing a Prominent CSF/Fluid Collection (Black Arrows) Following ID. The Site of Intradural Communication is Marked by White Arrow. Patient was Treated with Dural Sealant but Without Flat Bed Rest with Good Outcome. Follow-up T2 Sagittal (c) and Axial (d) Images (below) Showing Significantly Reduced CSF Collection (Black Arrows in c and d).



Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-124

**Figure 5:** CSF Leak Following ID. T2 Sagittal (a) Axial (b) Images (Above) Showing a Large CSF Collection (Black arrows). Site of Communication Shown by White Arrow in (b). Initially Patient had an Unsatisfactory Outcome Following Conservative Treatment. Subsequently Treated with Glue Injection. T2 Sagittal (c) and Axial (d) Images (Below) Showing Significantly Reduced Collection (Black Arrow in d) Following Glue Injection.




niques might be challenging to say the least.

Different methods for achieving satisfactory dural closure exist, with promising results of the advancing technology for dural sealing agents especially for durotomies where primary suturing is not thought to be feasible or technically possible without significant collateral damage.

Long periods of flat bed rest does not decrease the rate of complications, in fact early patients mobilisation potentially reduces the length of hospital stay, medical complications and the overall cost on the health system.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Wang JC, Bohlman HH, Riew KD. The management of long-term results of patients with dural tears from lumbar spine surgery. Presented at the annual meeting of the North American Spine Society, New York, USA October 22-25, 1997.

2. Wood GW. Lower back pain and disorders of intervertebral disc. In: Canale ST, ed. *Campbell's Operative Orthopaedics*. 9th ed. St. Louis, MO, USA: Mosby; 1998: 3014-3092.

3. Grannum S, Patel MS, Attar F, Newey M. Dural tears in primary decompressive lumbar surgery. Is primary repair necessary for a good outcome? *Eur Spine J*. 2014; 23(4): 904-908. doi: 10.1007/s00586-013-3159-9

4. Raskin NH. Lumbar puncture headache: A review. *Headache*. 1990; 30(4): 197-200. doi: 10.1111/j.1526-4610.1990.hed3004197.x

5. Eismont FJ, Wiesel SW, Rothman RH. Treatment of dural tears associated with spinal surgery. *J Bone Joint Surg Am*. 1981; 63: 1132-1136.

6. Hodges, Scott D, Eck JC, Covington LA. Management of incidental durotomy without mandatory bed rest: A retrospective review of 20 cases. *Spine*. 1999; 24(19): 2062-2064.

7. Low JC, von Niederhäusern B, Rutherford SA, King AT. Pilot study of perioperative accidental durotomy: Does the period of postoperative bed rest reduce the incidence of complication? *Br J Neurosurg*. 2013; 27(6): 800-802. doi: 10.3109/02688697.2013.798858

8. Than KD, Wang AC, Etame AB, La Marca F, Park P. Postoperative management of incidental durotomy in minimally invasive lumbar spinal surgery. *Minim Invasive Neurosurg*. 2008; 51: 263-266. doi: 10.1055/s-0028-1082313

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-124

9. Bonanos G, Coulter IC, Prasad M. Bed rest and beyond: An audit of bed rest for managing incidental durotomy in lumbar spine surgery. In: *Proceedings of the 2015 Autumn Meeting of the Society of British Neurological Surgeons*. *Br J Neurosurg*. 2015; 29(4): 472-473.

10. Clajus C, Stockhammer F, Rohde V. The intra- and post-operative management of accidental durotomy in lumbar spine surgery: Results of a German survey. *Acta Neurochir*. 2015; 157(3): 525-530. doi: 10.1007/s00701-014-2325-0

11. Gautschi OP, Stienen MN, Smoll NR, Corniola MV, Tessitore E, Schaller K. Incidental durotomy in lumbar spine surgery—a three-nation survey to evaluate its management. *Acta Neurochir*. 2014; 156(9): 1813-1820. doi: 10.1007/s00701-014-2177-7

12. Radcliff KE, Sidhu GD, Kepler CK. Complications of flat bed rest after incidental durotomy. *Clin Spine Surg*. 2016; 29(7): 281-284. doi: 10.1097/BSD.0b013e31827d7ad8

NEURO

Openventio PUBLISHERS

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-125

*Systematic Review*

***Corresponding author***
**Avinash Kumar Kanodia, MD, DM, FRCR**
Department of Radiology
Ninewells Hospital
Dundee, UK
**E-mail:** avinash.kanodia@nhs.net

***Volume 4 : Issue 1***
***Article Ref. #: 1000NOJ4125***

***Article History***
**Received:** April 26th, 2017
**Accepted:** May 16th, 2017
**Published:** May 16th, 2017

***Citation***
Abdelsadg M, Kanodia AK, Abbas A, Sheikh A. Chronic subdural haematoma: Systematic review highlighting risk factors for recurrent bleeds. *Neuro Open J*. 2017; 4(1): 16-24. doi: 10.17140/NOJ-4-125



# Chronic Subdural Haematoma: Systematic Review Highlighting Risk Factors for Recurrent Bleeds

**Mohamed Abdelsadg, MBBS, PgDip[1]; Avinash Kumar Kanodia, MD, DM, FRCR[2*]; Athar Abbas, MBBS[1]; Asim Sheikh, FRCS[3]**

*[1]Department of Neurosurgery, Ninewells Hospital, Dundee, UK*
*[2]Department of Radiology, Ninewells Hospital, Dundee, UK*
*[3]Department of Neurosurgery, Leeds General Infirmary, Leeds, UK*

## ABSTRACT

**Introduction:** Chronic subdural haematoma (CSDH) is one of the commonest forms of intracranial haemorrhage. Surgical drainage of CSDH is a routine operation in the modern neurosurgical practice which has shown to be the most effective way in treating this entity; however, the incidence of recurrence of the haematoma post operatively remains as high as 26.5%. The risk factors for CSDH recurrence remains an area of ongoing research.
**Objective:** We have conducted a systematic review to evaluate the available literature addressing the risk factors for CSDH recurrence, aiming to minimise or at least identify patients at higher risk of recurrence in order to decrease associated morbidity.
**Methods:** Ovid *via* Medline, PubMed, and Google scholar databases were searched for eligible studies, search results were then limited to studies in English language, Humans and studies published within the last 5 years. The included studies were critically appraised using the Critical Appraisal Skills Programme (CASP) tool, and each study has then been ranked using the Harbour and Miller hierarchy of ranking.
**Results:** Based on available evidence, we classified the risk factors associated with recurrence to patients', radiological, and surgical factors. Patient factors include history of seizures, trauma, alcoholism, brain atrophy, and presence of CSF shunts, while the role of diabetes in relation to the recurrence is controversial. Radiologically the presence of air in the subdural space post-operatively, the width of the haematoma, and the presence of bilateral CSDHs are associated with increased risk of recurrence. While the predictive value of multiple membranes in the CSDH remains controversial. Surgically, the risk of recurrence was noted to be higher in patients with parietal or occipital compared to those who had frontal burr hole drainage, also placing a subdural drain decreases the chance of recurrence and some evidence showed better outcomes for frontally placed drains. The role of anti-inflammatory agents (including steroids) remains an area of ongoing debate.
**Conclusions:** Risk factors for CSDH can be divided into patients', radiological, and surgical factors. We encourage health care providers to minimize if not prevent potentially avoidable factors. Patients with increased risks for recurrence should be identified early by the treating team and when possible should be informed about their higher than usual risk of recurrence. Moreover this review highlights the general lack of a sufficiently powered class I evidence addressing this topic and that further research is required in this topic.

**KEY WORDS:** Chronic subdural haematoma; Recurrence; Bur hole drainage; Outcome.

**ABBREVIATIONS:** CSDH: Chronic subdural haematoma; CASP: Critical Appraisal Skills Programme; CT: Computed Tomography; DM: Diabetes Mellitus.

## INTRODUCTION

Chronic subdural haematoma (CSDH) is one of the commonest forms of intracranial haemor-

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

rhage. Surgical drainage of CSDH is a routine operation in the modern neurosurgical practice.[1,2] The incidence of CSDH is 8-58 per 100,000 in individuals over 65 years of age.[3] However, with continuous rise of life expectancy together with a widening usage of anti-coagulants and anti-platelets medications worldwide, the incidence of this is likely to continue rising.[4] Clinically, as the name suggests, CSDH does not present acutely and it may remain silent for variable periods of times and may present insidiously or with non-specific features. Surgical intervention has been shown to be the most effective way in treating this entity; however, the incidence of recurrence ranges from 9.2-26.5%.[1,2,5-7] The recurrence can also remain silent with delay in diagnosis and associated morbidity and mortality.

We have conducted this Systematic review to evaluate the available literature addressing the risk factors for CSDH recurrence, aiming to minimise or at least identify patients at higher risk of recurrence in order to decrease associated morbidity. Moreover, this Systematic review will address areas where further research is required to provide robust evidence in the topic, as the implementation of evidence based medicine provides high quality standard medical care at the lowest cost.[8]

A brief analysis of the literature will be conducted using the Critical Appraisal Skills Programme (CASP) tool[9] and then each study will be ranked using the Harbour and Miller[10] hierarchy of ranking. See appendix I.

### Search Strategy

Table 1 below summarises the search strategy used for the literature search.

Ovid *via* Medline, PubMed and google scholar search resulted in 33 publications, which was then limited to studies in English language, Humans and the duration between 2012 and current date this limited the publications to 18 papers.

Following is a justification for the used search limitation:

English Language is an international language for healthcare, the majority of the top journals with high impact factors are in English, and is the most widely learned second language. Nevertheless, we are aware that by limiting the search to publications in a single language this could potentially affect the generalisability and possibly results in English language, selection, publication, and citation biases.[11] The search was also limited to humans, given the limited role for the animal derived data in this topic.

With regards to publication period this was limited to the last 5 years to ensure contemporaneous evidence. Nevertheless, following the search, studies titles and abstracts were screened for relevance, and reference lists of included papers were reviewed with 'backward chaining' employed to include seminal papers. Following limiting the search to the above, 18 studies were screened, and limited by the type of this review only 6 papers will be discussed.

### Review of Literature

CSDH is one of the most commonly encountered conditions in neurosurgery; however, there is no consensus regarding clinical features, correlating factors, or causes of recurrence.[12] Clinically, recurrent bleed can also be challenging and both clinical and imaging factors can be used to make a positive diagnosis. Moreover, presence of a rebleed does not always result in repeat surgery and similarly, significant rebleed may remain clinically

**Table 1:** Search Strategy.

| | |
|---|---|
| Keywords | The following key words were set to be recognised within article title, abstract, and/or keywords:<br>Subdural Hematoma, chronic subdural haematoma, recurrence, risk factors |
| Search terms | -Chronic subdural haematoma (OR) subdural haematoma<br>-Recurrence (AND) risk factors<br>-Chronic subdural haematoma (OR) subdural haematoma, (AND) recurrence (AND) risk factors |
| Limitations | The search was limited to the following:<br>English Language.<br>Humans.<br>Between 2012 and current date. |
| Inclusion criteria | The search included patients with chronic subdural haematoma (unilateral or bilateral, surgically or non-surgically managed)<br>The search also included systematic reviews, RCTs, cohort studies, and literature reviews. |
| Exclusion criteria | The search excluded:<br>Solely pregnant and post-partum patients.<br>Neonates and paediatrics.<br>Case reports.<br>Descriptive reports. |
| Databases used | Ovid SP (MedLine/Embase), PubMed, Google Scholar |
| Screening evidenced | Following the search, studies titles and abstracts were screened for relevance, inclusion and exclusion criteria, and non-qualifying articles were then excluded. Reference lists of included papers were reviewed with 'backward chaining' employed to gather pertinent papers for consideration. |
| Final number | 6 studies will be addressed |

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

silent and undiagnosed for variable periods of time, potentially with adverse outcomes. It is therefore important that the risk factors associated with rebleeding are identified and such patients are observed and followed-up more closely. Multiple studies have been conducted to identify potential risk factors contributing to the pathogenesis of CSDH and its recurrence with numerous factors reported.[12-22]

Yamamoto et al[13] attempted to determine independent predictors contributing to the recurrence of chronic subdural hematoma (CSDH) in 105 patients who underwent CSDH surgery over 9 years period, with follow-up computed tomography (CT) scanning performed 1 day, 1 week, 1 month, 3 months, and 6 months post-operatively. The criteria used to define recurrence were radiological; however, clinical recurrence (prompted by re appearance of symptoms) warranted earlier scanning. The radiological recurrence was an increase in the hematoma thickness and a change in hematoma density on follow-up CT scans within 3 months post-operatively.[13] By using univariate and multivariate analyses to assess the relationships among various variables and CSDH recurrence Yamamoto et al[13] reported four independent variables affect the recurrence of CSDH: a positive history of seizures and the width (maximum diameter) of the hematoma were positively associated with increased risk of recurrence, while a positive history of diabetes mellitus (DM) and the multiplicity of hematoma cavities (multiple membrane) on CT scans were both associated with less risk for recurrence. Brief discussion regarding these factors will follow.[13] However, the aforementioned study was a retrospective cohort study, and thus is potentially subject to sources of bias and variation. The sample size of the study was limited, and when considering the incidence of the disease the study will be under-powered; hence, further investigation is required to assess the independent predictors revealed in the study. The study was therefore scored 2+ in the Harbour and Miller[10] hierarchy of ranking.

Several studies support the role of seizure disorders, alcoholism, cerebrospinal fluid shunts, anticoagulation therapy and coagulopathies.[13,23-25] These may be variably associated with head trauma, brain atrophy and decreased blood homeostasis. Seizures can be associated to the recurrence of CSDH due to the occasional head injury associated with certain types of seizures, or as a result of coagulopathy due to some anticonvulsants or to their effect on the liver causing disruption of the coagulation cascade.[26]

While the width of the hematoma is often determined at the level of the maximum thickness of the clot, it has been reported to be associated with the patient age, with the underlying atrophy of the aging brain providing the space for the hematoma to grow and/or recur.[27] This may also lead to poor brain re-expansion after the operation. Poor brain re-expansion has been correlated with recurrence in previous reports.[19,28]

Hyperglycaemia secondary to diabetes mellitus is associated with vascular occlusive disorder secondary to the hyperviscosity of the blood and the often encountered atherosclerosis.[29] Yamamoto et al[13] suggested that DM may play a role in decreasing the re-bleeding tendency of CSDH, since patients with DM has a high osmotic pressure and increased platelet aggregation.[29] This theory could be supported by the findings of previous study which reported that osmotherapy performed using 20% mannitol is effective in stopping repeated bleeding of a CSDH.[30] On the other hand capillary vasculopathy including haemorrhage (e.g., retinal haemorrhage) is one of the major complications in diabetic patients, and the exudation from the capillaries in the membrane of CSDH plays an important role in its enlargement.[31,32] Moreover, similar study showed increased but non-significant risk for recurrence in patients with DM, it was also found that patients with bilateral CSDH tend to have DM.[17]

With regards to multiplicity of the hematoma cavity conflicting reports were published. While previous studies reported multiplicity to be positively correlated with recurrence of CSDH,[2,33] other concluded it is associated with lower rates of recurrence.[13] This conflicting evidence could be attributed at least partially to the discrepancy in defining "multiplicity of the haematoma". In some studies with a positive correlation, the authors identified "multiplicity" as multiple CSDHs,[2,33] whereas Yamamoto et al defined multiplicity of hematoma cavities as the involvement of multiple cavities, similar to what has been previously described as trabecular haematoma (Figure 1).[5,13]

Torihashi et al[17] conducted a study to determine independent predictors associated with CSDH recurrence. The results demonstrated that bilateral CSDH was an independent risk factor for the recurrence of CSDH. Although, anti-platelet and anticoagulant therapy had no statistically significant effect on CSDH recurrence, the time interval between the injury and the first operation for patients with anti-platelet and/or anti-coagulant therapy was shorter (29.9 *vs.* 44.2 days).[17] The relative strengths of the above study were the bigger sample size and the fact they used a logistic regression model in performing a multivariate statistical analysis of the recurrence factors. Nonetheless being a retrospective study it scores 2++ in the Harbour and Miller hierarchy of ranking.[10] Further studies also supported bilateral CSDH as a risk factor for recurrence (Figure 2).[18,19] It is though that patients with bilateral CSDH tend to have previous brain atrophy increasing the risks of recurrence as discussed earlier.

Abouzari et al[20] conducted a study looking at the role of posture in post-operative patients in the recurrence of surgically managed traumatic CSDH.[20] The study concluded that assuming an upright posture soon after burr-hole surgery is associated with an increased incidence of CSDH recurrence. Another study showed similar but statistically non-significant higher recurrence rate of CSDH with early sitting up posture in comparison to 3 days of bed rest.[34] The limitations of Abouzari et al[20] study was that they only studied patients with a history of head trauma and excluded those with shunts, seizures, alcohol abuse or use of anticoagulants. While up to 40% of patients with CSDH cannot recall a history of trauma,[35] this very homogenous study group

NEURO
Open Journal

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

**Figure 1:** CT Scans of 2 Different Patients (a,b) and (c,d) Show Multiplicity of Haematoma on Right Side with Blood Products of Different Attenuations along with Presence of a "Membrane" (White Arrow).

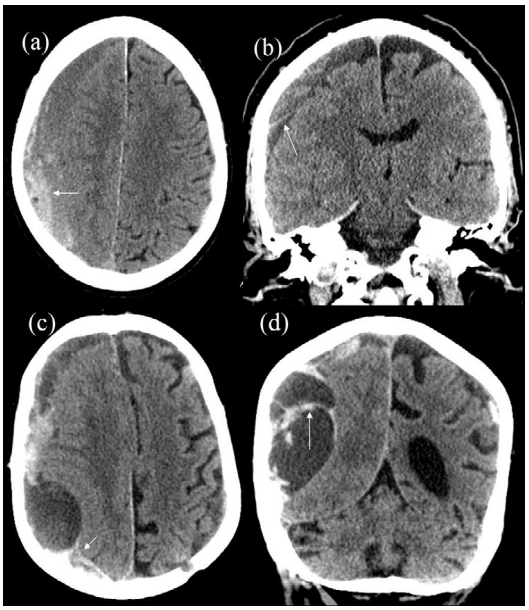
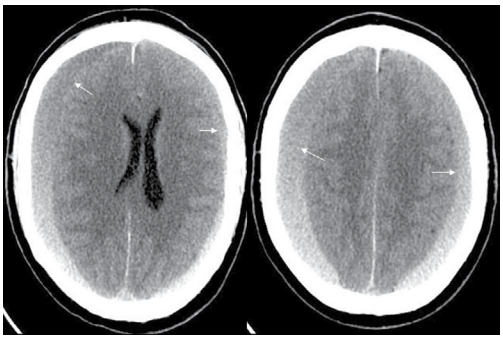



**Figure 2:** CT Scan Showing Presence of Bilateral Subdural Haematomas (Arrows).



in the Abouzari et al[20] trial brings the generalizability of the trial into question. In the same study recurrence was defined by radiological criteria, and despite the radiological recurrence rate was significantly higher in the patients who assumed a head-elevated position immediately after surgery, these recurrences did not seem to affect the patients' clinical recovery and only one patient required surgery to drain the recurrent haematoma,[20] the study was inadequately powered and no details for statistical analysis was included, therefore scored 1 in the Harbour and Miller hierarchy of ranking.[10]

Another study looking at the "radiological factors" associated with risks of CSDH recurrence, showed increased risk of rebleed in patients with parietal or occipital drainage compared to those who had frontal burr hole drainage. It also showed that patient with residual subdural air on CT scans obtained 7 days post-surgery had a higher recurrence rate than those with no subdural air on the CT scan (Figure 3).[12] Similar observation was drawn by Nagata et al[36] showing that the amount of subdural air found postoperatively correlated negatively with the resolution rate of CSDH.

To further explain the effect of different risk factors, different theories have been proposed to explain post-operative recurrence of CSDH. One is the pressure difference theory which emphasises pressure imbalance between the outside and inside of the inner haematoma membrane (subdural space and the subarachnoid/subpial space); that is high pressure in the hematoma cavity and/or low pressure in the subarachnoid space (Figure 4). The earlier situation is indicated by massive subdural air collection, residual SDH and persistent widening of the hematoma cavity (ongoing bleeding in the subdural space). The latter situation is indicated by excessive fluid loss such as dehydration, anemia, excessive cerebrospinal fluid drainage or impact of se-

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

**Figure 3:** CT Scans Showing Significant Amount of Air in the Subdural Cavities on both Sides Post Drainage (White Arrows).

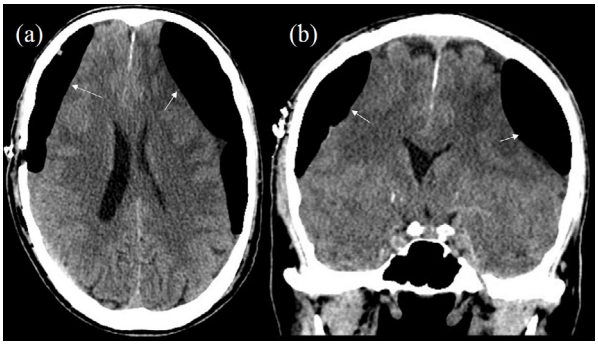
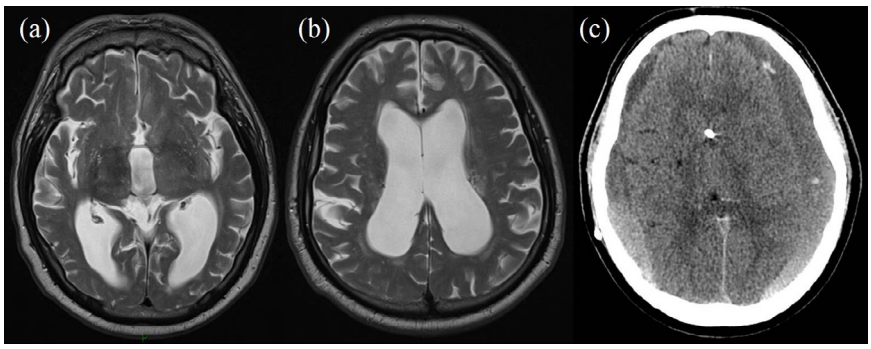



**Figure 4:** MRI Scan (a, b) Shows Dilated Ventricular System in a Patient Clinically Presenting with Normal Pressure Hydrocephalus. Post Drainage, CT (c) Shows Bilateral Subdural Haematomas with a Decompressed Ventricular System.




vere brain atrophy.[35,37] Moreover, Nakaguchi et al[12] also reported that patients with a subdural space more than 10 mm wide on CT scans obtained 7 days post-surgery had a higher recurrence rate than those with a space measuring 10 mm or less. The study concluded that post-operative re-accumulation of CSDH can be reduced by placing the tip of the drainage catheter in the frontal convexity and by removing subdural air during or after surgery.[12] This is explainable by the fact that air accumulates in the frontal convexity while the patient is supine immediately after surgery. With the same principle in mind for draining extra fluid and air from the subdural space, Cambridge conducted a randomised trial of using a subdural drain *versus* no drain following evacuation of CSDH concluded that the use of a drain after burr-hole drainage of chronic subdural haematoma is safe and associated with reduced recurrence and mortality at 6 months.[38] Nakaguchi et al scored 2+ in the in the Harbour and Miller hierarchy of ranking, while the Cambridge trial scores 1+ as a well conducted randomised controlled trial with low risk of bias.[10,38]

Another theory is the inflammatory theory, first proposed In 1857 by Virchow who described CSDH as a dural inflammatory disease and called it "pachymeningitis hemorrhagica interna." He was the first to stress the importance of inflammation for the onset and development of CSDH.[39] Later on several studies demonstrated that CSDH is the result of a local inflammatory reaction of the dura to an injurious stimulus.[35,40] Frati et al[21] conducted a prospective study over 2 years period to determine role of local inflammation in the pathogenesis and post-operative recurrence of chronic subdural hematoma (CSDH).[21] The study - although significantly under powered - has included only patients who can clearly recall history of head trauma, and showed evidence of an inflammatory process within the dural border cell layer, this has a clear impact on the generalisability of the trial, scoring 2+ in the Harbour and Miller hierarchy of ranking.[10] The study concluded that higher levels of inflammatory cytokines were positively correlated with recurrence and re-accumulation of the CSDH. Frati et al advocated for a prolonged post-operative course of anti-inflammatory medicine given as prophylaxis to minimise the risks of CSDH recurrence. Similar rationale and conclusion were reached by another recently published study advocating the use of steroids following the surgical evacuation of CSDH to prevent recurrence.[22] The role of steroids in CSDH remains a controversial topic; nonetheless, an ongoing trial in the UK is currently addressing this and hopefully will put an end to this debate.[41]

Most recently the British Neurosurgical Trainee Research Collaborative (BNTRC) published the largest multicenter, prospective, observational cohort study looking at the management and outcome for patients with chronic subdural

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

Table 2: Summary of the Studies Discussed in this Paper, the Aim of Each, Concluded Factors for CSDH Recurrence, Strengths, Weaknesses, and Score in the Harbour and Miller hierarchy of ranking.[10]

| Article | Aim of study | Factors associated with increased risk for CSDH recurrence | Strengths | Weaknesses | Score |
|---|---|---|---|---|---|
| Yamamoto et al[2] | To determine independent predictors contributing to the recurrence of CSDH | - Width of the hematoma.<br>- Multiplicity of hematoma cavities.<br>- Seizures<br>- Negative history of DM | - Clear definition for recurrence<br>- Robust statistical analysis | - Retrospective<br>- Small sample size | 2+ |
| Torihashi et al[21] | To determine independent predictors contributing to the recurrence of CSDH | - Bilateral CSDH | - Larger sample size<br>- Robust statistical analysis. | - Retrospective | 2++ |
| Abouzari et al[27] | To evaluate the relationship between recurrence rate of CSDH and patient posture postoperatively | - Assuming an upright posture soon after burr-hole surgery | - Randomized double linded controlled trial | -Generalizability<br>- Underpowered<br>- Radiologically defined recurrence with very limited clinical sequel | 1- |
| Nakaguchi et al[8] | To determine features of CSDHs recurrence rate on the basis of the natural history of these lesions and their intracranial extension | - Subdural space more than 10 mm wide on CT 7 days post-surgery<br>- Subdural drain not placed on the frontal convexity<br>- Presence of subdural air, intra or post operatively<br>- Cranial base type of CSDHs was high | - Prospective study<br>- Over 9 years<br>- Long term follow-up | - Single center<br>- Small sample size<br>- Recurrence defined radiologically with no clinical correlation | 2+ |
| Frati et al[34] | To determine role of local inflammation in the pathogenesis and recurrence of CSDH | - Higher levels of inflammatory cytokines | - Prospective study | - Under powered<br>- Generalizability | 2+ |
| Brennan et al[42] | To examine the management and outcome for patients with CSDH across the UK | - Failure to insert a drain intraoperatively | - Multicenter<br>- Prospective<br>- Clear definition for recurrence | - Lack of long term follow-up<br>- Skewed to single surgical drainage technique | 2++ |

CSDH: Chronic subdural haematoma; CT: Computed Tomography.

haematomas.[42] This has included centres throughout the United Kingdom (UK) and Ireland, and showed the rates of CSDH mortality (2%), symptomatic recurrence (9%), and unfavorable functional outcome (22%) were all acceptable when audited against predefined criteria from the literature.[42] However, multivariate analysis demonstrated that failure to insert a drain intraoperatively independently predicted recurrence ($p$=0.011) as well as unfavorable functional outcome ($p$=0.048). Reinforcing previous studies conclusions, the BNTRC group detected statistically significant unfavorable functional outcomes following prescribed post-operative bed rest ($p$=0.019).[20,35] It also concluded that Increasing patient age ($p$<0.00001) is associated with unfavorable functional outcome; however, there was no significant difference in relation to recurrence, consolidating previous reports recommendations.[13]

Unlike previous studies; the BNTRC had clear definition to the recurrence of CSDH, which was clinical recurrence of CSDH symptoms, confirmed radiologically, and requiring surgery within 60 days.[42] On the other hand one of the study's limitations was the lack of long term follow as patients were observed only during their admission course at the neurosurgical unit (NSU). Moreover, the study cohort was skewed to single surgical drainage technique (burr hole drainage) which was the modality used in 89% of operated cases; hence, making predicting outcome in patients treated with other surgical techniques (e.g., mini craniotomy) an area of ongoing debate.

The study nevertheless was well conducted and scores 2++ in the in the Harbour and Miller hierarchy of ranking (Table 2).

## CONCLUSION

The review highlights the lack of unified definition for CSDH recurrence as different studies use different methods in labelling recurrence; nonetheless the majority combine clinical features as well as imaging modalities to identify recurrence of CSDH. The available evidence is generally underpowered and more research is required in this topic.

There are different factors contributing to the recurrence of CSDH, which can be divided into patient factors, radiological factors, surgical/technical factors, and post-operative factors.

Patient factors include history of seizures, trauma, alcoholism, brain atrophy, and presence of CSF shunts, while there is conflicting evidence regarding the role of DM in relation to recurrence risk of CSDH.

Radiological factors include presence of air in the subdural space in the post-operative scan, width of the haematoma, width of the subdural space and presence of bilateral CSDH. The predictive value of presence of multiple membranes in the

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

CSDH remains controversial.

With regards to the surgical factors, there are different techniques adopted, nonetheless it was found that burr hole craniotomy is the most adopted method, and there is lack of evidence testing outcomes of other surgical techniques. The risk of CSDH recurrence is higher in patients with parietal or occipital drainage compared to those who had frontal burr hole drainage. Placing a subdural drain was noted to decrease the chance of recurrence and some evidence showed better outcomes for frontally placed drains.

Post-operative patient positioning seems to affect the recurrence risk, with the current evidence promoting avoidance of early sitting up of patients with CSDH. It is clearly noted that more studies are necessary to address this topic.

The role of anti-inflammatory agents (including steroids) remains an area of hot debate. There is a need of well conducted adequately powered multicentre randomised trial(s) to increase our understanding and deliver more robust recommendation regarding the topic .

Finally, we have briefly described the factors thought to be associated with increased risk of recurrent CSDH and highlighted areas of ongoing debate.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interst.

## REFERENCES

1. Asano Y, Hasuo M, Takahashi I, Shimosawa S. Recurrent cases of chronic subdural hematoma--its clinical review and serial CT findings. *No to Shinkei*. 1992; 44(9): 827-831.

2. El-Kadi H, Miele VJ, Kaufman HH. Prognosis of chronic subdural hematomas. *Neurosurg Clin N Am*. 2000; 11: 553-556.

3. Ducruet AF, Grobelny BT, Zacharia BE, et al. The surgical management of chronic subdural hematoma. *Neurosurg Rev*. 2012; 35: 155-169. doi: 10.1007/s10143-011-0349-y

4. Aspegren OP, Astrand R, Lundgren MI, Romner B. Anticoagulation therapy a risk factor for the development of chronic subdural hematoma. *Clin Neurol Neurosurg*. 2013; 115: 981-984. doi: 10.1016/j.clineuro.2012.10.008

5. Nakaguchi H, Tanishima T, Yoshimasu N. Factors in the natural history of chronic subdural hematomas that influence their postoperative recurrence. *J Neurosurg*. 2001; 95: 256-262.

6. Wakai S, Hashimoto K, Watanabe N, Inoh S, Ochiai C, Nagai M. Efficacy of closed-system drainage in treating chronic subdural hematoma: Aprospective comparative study. *Neurosurgery*. 1990; 26: 771-773.

7. Zumkeller M, Höllerhage HG, Dietz H. Treatment outcome in patients with chronic subdural hematoma with reference to age and concurrent internal diseases [In German]. *Wien Med Wochenschr*. 1997; 147: 55-62.

8. Lewis S, Orland B. The importance and impact of evidence-based medicine. *J Manag Care Pharm*. 2004: 10(5 Suppl A): 3-5. doi: 10.18553/jmcp.2004.10.S5-A.S3

9. Critical Appraisal Skills Programme (CASP). 2010 Tools. [Online]. Web site. http://www.casp-uk.net/#!checklists/cb36. Accessed April 19, 2017.

10. Harbour R, Miller J. Education and debate: A new system for grading recommendation in evidence based guidelines. *BMJ*. 2001: 323: 334-336.

11. Egger M, Smith GD. Bias in location and selection of studies. *BMJ*. 1998; 316(7124): 61-66.

12. Nakaguchi H, Tanishima T, Yoshimasu N. Relationship between drainage catheter location and postoperative recurrence of chronic subdural hematoma after burr-hole irrigation and closed-system drainage. *J Neurosurg*. 2000; 93(5): 791-795. doi: 10.3171/jns.2000.93.5.0791

13. Yamamoto H, Hirashima Y, Hamada H, Hayashi N, Origasa H, Endo S. Independent predictors of recurrence of chronic subdural hematoma: Results of multivariate analysis performed using a logistic regression model. *J Neurosurg*. 2003; 98(6): 1217-1221. doi: 10.3171/jns.2003.98.6.1217

14. Foelholm R, Waltimo O. Epidemiology of chronic subdural haematoma. *Acta Neurochir*. 1975; 32: 247-250. doi: 10.1007/BF01405457

15. Fogelholm R, Heiskanen O, Waltimo O. Chronic subdural hematoma in adults. Influence of patient's age on symptoms, signs, and thickness of hematoma. *J Neurosurg*. 1975; 42: 43-46. doi: 10.3171/jns.1975.42.1.0043

16. Fukuhara T, Gotoh M, Asari S, et al. The relationship between brain surface elastance and brain reexpansion after evacuation of chronic subdural hematoma. *Surg Neurol*. 1996; 45: 570-574. doi: 10.1016/0090-3019(95)00471-8

17. Torihashi K, Sadamasa N, Yoshida K, Narumi O, Chin M, Yamagata S. Independent predictors for recurrence of chronic subdural hematoma: A review of 343 consecutive surgical cases. *Neurosurgery*. 2008; 63(6): 1125-1129. doi: 10.1227/01.NEU.0000335782.60059.17

18. Probst C. Peritoneal drainage of chronic subdural hematomas in older patients. *J Neurosurg*. 1988; 68: 908-911. doi:

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

10.3171/jns.1988.68.6.0908

19. Robinson RG. Chronic subdural hematoma: Surgical management in 133 patients. *J Neurosurg*. 1984; 61: 263-268. doi: 10.3171/jns.1984.61.2.0263

20. Abouzari M, Rashidi A, Rezaii J, et al. The role of postoperative patient posture in the recurrence of traumatic chronic subdural hematoma after burr hole surgery. *Neurosurgery* 2007; 61(4): 794-797. doi: 10.1227/01.NEU.0000298908.94129.67

21. Frati A, Salvati M, Mainiero F, et al. Inflammation markers and risk factors for recurrence in 35 patients with a posttraumatic chronic subdural hematoma: A prospective study. *J Neurosurg*. 2004. 100(1) 24-32. doi: 10.3171/jns.2004.100.1.0024

22. Qian Z, Yang D, Sun F, Sun Z. Risk factors for recurrence of chronic subdural hematoma after burr hole surgery: Potential protective role of dexamethasone. *Br J Neurosurg*. 2017; 31(1): 84-88. doi: 10.1080/02688697.2016.1260686

23. Nomura S, Kashiwagi S, Fujisawa H, et al. Characterization of local hyperfibrinolysis in chronic subdural hematomas by SDSPAGE and immunoblot. *J Neurosurg*. 1994; 81: 910-913. doi: 10.3171/jns.1994.81.6.0910

24. Oishi M, Toyama M, Tamatani S, et al. Clinical factors of recurrent chronic subdural hematoma. *Neurol Med Chir.* 2001; 41: 382-386. doi: 10.2176/nmc.41.382

25. Park CK, Choi KH, Kim MC, et al. Spontaneous evolution of posttraumatic subdural hygroma into chronic subdural haematoma. *Acta Neurochir*. 1994; 127: 41-47. doi: 10.1007/BF01808545

26. So CC, Wong KF. Valproate-associated dysmyelopoiesis in elderly patients. *Am J Clin Pathol*. 2002; 118: 225-228. doi: 10.1309/4TEF-LVGX-WEQ9-R8W8

27. Foelholm R, Waltimo O. Epidemiology of chronic subdural haematoma. *Acta Neurochir*. 1975; 32: 247-250. doi: 10.1007/BF01405457

28. Mori K, Maeda M. Surgical treatment of chronic subdural hematoma in 500 consecutive cases: Clinical characteristics, surgical outcome, complications, and recurrence rate. *Neurol Med Chir (Tokyo)*. 2001; 41: 371-381. doi: 10.2176/nmc.41.371

29. Kernan WN, Inzucci SE, Viscoli CM, et al. Insulin resistance and risk for stroke. *Neurology*. 2002; 59: 809-815. doi: 10.1212/WNL.59.6.809

30. Suzuki J, Takaku A. Nonsurgical treatment of chronic subdural hematoma. *J Neurosurg*. 1970; 33(5): 548-553. doi: 10.3171/jns.1970.33.5.0548

31. Ito H, Komai T, Yamamoto S. Fibrinolytic enzyme in the lining walls of chronic subdural hematoma. *J Neurosurg*. 1978; 48: 197-200. doi: 10.3171/jns.1978.48.2.0197

32. Tokmak M, Iplikcioglu AC, Bek S, Gökduman CA, Erdal M. The role of exudation in chronic subdural hematomas. *J Neurosurg*. 2007; 107: 290-295. doi: 10.3171/JNS-07/08/0290

33. Tanikawa M, Mase M, Yamada K, et al. Surgical treatment of chronic subdural hematoma based on intrahematomal membrane structure on MRI. *Acta Neurochir*. 2001; 143: 613-619. doi: 10.1007/s007010170067

34. Nakajima H, Yasui T, Nishikawa M, Kishi H, Kan M. The role of postoperative patient posture in the recurrence of chronic subdural hematoma: A prospective randomized trial. *Surg Neurol*. 2002; 58: 385-387. doi: 10.1016/S0090-3019(02)00921-7

35. Markwalder TM. Chronic subdural hematomas: A review. *J Neurosurg*. 1981; 54: 637-645. doi: 10.3171/jns.1981.54.5.0637

36. Nagata K, Asano T, Basugi N, et al. Studies on the operative factors affecting the reduction of chronic subdural hematoma, with special reference to the residual air in the hematoma cavity. *No Shinkei Geka*. 1989;17: 15-20.

37. Smyth H, Livingston K. Ventricular infusion in the operative management of subdural hematoma. In: Morley T, ed. *Current Controversies in Neurosurgery*. Philadelphia, USA: WB Saunders; 1976: 566-571.

38. Santarius T, Kirkpatrick PJ, Ganesan D et al. Use of drains versus no drains after burr-hole evacuation of chronic subdural haematoma: A randomised controlled trial. *The Lancet*. 2009; 374(9695): 1067-1073. doi: 10.1016/S0140-6736(09)61115-6

39. Virchow R. Das Hamaton der dura mater [In German]. *Verch Phys Med Ges Wurzburg*. 1857; 7: 134-142

40. Markwalder TM, Steinsiepe KF, Rohner M, et al. The course of chronic subdural haematoma after burr-hole craniostomy and closed-system drainage. *J Neurosurg*. 1981; 55: 390-396. doi: 10.3171/jns.1981.55.3.0390

41. DEXamethasone in Chronic SubDural Haematoma (Dex-CSDH trial). A randomised, double blind, placebo-controlled trial of a two-week course of dexamethasone for adult patients with a symptomatic chronic subdural haematoma. Web site. http://www.dexcsdh.org/. Accessed Appril 02, 2017.

42. Brennan PM, Kolias AG, Joannides AJ, et al. The management and outcome for patients with chronic subdural hematoma: A prospective, multicenter, observational cohort study in the United Kingdom. *J Neurosurg*. 2017: 1-8. Ahead of print. doi: 10.3171/2016.8.JNS16134

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-125

**Appendix I**

### Harbour and Miller hierarchy of evidence[10]

1++ High quality meta analyses, systematic reviews of RCTs or RCTs with a very low risk of bias.
1+ Well conducted meta analyses, systematic reviews of RCTs, or RCTs with a low risk of bias.
1- Meta analyses, systematic reviews of RCTs, or RCTs with a high risk of bias.
2++ High quality systematic reviews of case-control or cohort studies. High quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is casual.
2+ Well conducted case-control or cohort studies with a low risk of confounding, bias or chance and a moderate probability that the relationship is casual.
2- Case-control or cohort studies with a high risk of confounding, bias, chance and a significant risk that the relationship is not casual.
3 Non-analytic studies, e.g., case reports, case series.
4 Expert opinion.

NEURO

Openventio PUBLISHERS

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-126

Case Report

*Corresponding author
**Avinash Kumar Kanodia**, MD, DM, FRCR
Department of Radiology
Ninewells Hospital and Medical School
Dundee, UK
**E-mail:** avinash.kanodia@nhs.net

*Volume 4 : Issue 1*
*Article Ref. #: 1000NOJ4126*

***Article History***
**Received:** May 18th, 2017
**Accepted:** June 16th, 2017
**Published:** June 21st, 2017

***Citation***
Badran K, Abdelsadq M, Abbas A, et al. An unusual intracranial inflammatory process of unknown origin. *Neuro Open J*. 2017; 4(1): 25-30. doi: 10.17140/NOJ-4-126



# An Unusual Intracranial Inflammatory Process of Unknown Origin

**Khaled Badran, MBBS, MRCS (Ed)[1]; Mohamed Abdelsadq, MBBS, PG.Dip[1]; Athar Abbas, MBBS[1]; Jonathan O'Riordan, MD, FRCPE[2]; Avinash Kumar Kanodia, MD, DM, FRCR[3*]; Graham Leese, BMSc, MBChB, MD, FRCP, FRCPE[4]; Kismet Hossain-Ibrahim, FRCS, PhD[1]**

*[1]Department of Neurosurgery, Ninewells Hospital and Medical School, Dundee, UK*
*[2]Department of Neurology, Ninewells Hospital and Medical School, Dundee, UK*
*[3]Department of Radiology, Ninewells Hospital and Medical School, Dundee, UK*
*[4]Department of Endocrinology, Ninewells Hospital and Medical School, Dundee, UK*

## ABSTRACT

We present an unusual case of steroid responsive inflammatory condition, involving sellar suprasellar region with further ependymal lesions. This is complicated by previous surgery due to pituitary adenoma, not thought to related to inflammatory process. The patient responded well to steroids, but deteriorated due to development of hydrocephalus caused by obstruction due to adhesions. Despite extensive literature review and consideration of all known pathological conditions, it was concluded that the condition represented another inflammatory entity not yet fully characterised. The case also highlights that despite the steroid responsive nature of the condition, the ependymal involvement can result in progressive acute obstructive hydrocephalus with clinical deterioration. This case also suggests close follow-up and early imaging for early detection and treatment of this complication.

**KEY WORDS:** Intracranial langerhans cell histiocytosis; Lymphocytic hypophysitis.

**ABBREVIATIONS:** TB: Tuberculosis; LCH: Langerhans Cell Histiocytosis; LYH: Lymphocytic hypophysitis; ADEM: Acute disseminated encephalomyelitis; CDI: Central Diabetes Insipidus; HPA-LCH: Hypothalamic-pituitary axis change LCH; ND-LCH: Neurodegenerative change LCH; ECD: Erdheim-Chester Disease.

## CASE REPORT

This case report pertains to a 46-year-old male Caucasian who had endoscopic treatment for a pituitary adenona three years previously. He required replacement hydrocortisone and testosterone. Prior to the surgery, he was not known to have any other neurological issues.

A year after surgery following minor trauma, he underwent a computed tomography (CT) scan of the head. This confirmed no residual pituitary tumour (Figure 1). He was somewhat non-compliant with endocrine follow-up. Surveillance magnetic resonance imaging (MRI) imaging 2 years following initial surgery demonstrated post-up change with some enhancing tissue scaling the pituitary stalk and hypothalamic region; this was not clearly seen on the previous CT scan, possibly due to different modalities and absence of contrast imaging. Two months later, he developed polydipsia, fatigue and a partial left-sided homonymous visual field deficit. There was progressive confusion with fluent dysphasia and inattention. He was pyrexial. Gaze evoked nystagmus was present to the right. The rest of his examination was unremarkable.

MRI brain demonstrated significant progression of the abnormalities seen on the earlier scan with increased enhancing tissue involving the pituitary stalk, hypothalamic area and floor of the 3rd ventricle, also including the optic chiasm (Figure 2). Associated signal

NEURO
Open Journal

Openventio
PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-126

**Figure 1:** Initial CT Scan Performed at the Time of Head Injury. (a) Coronal and (b) Sagittal Reconstructions Show an Empty Sella (white arrows) with no Discernible Soft Tissue.

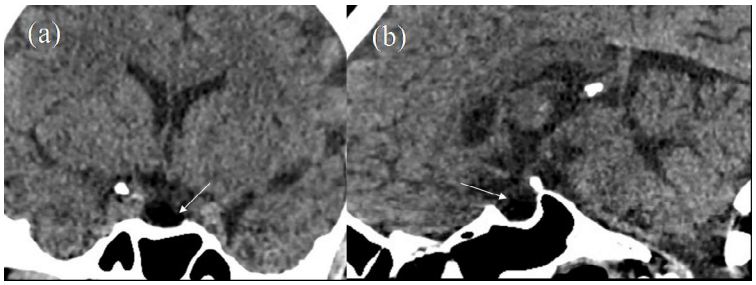
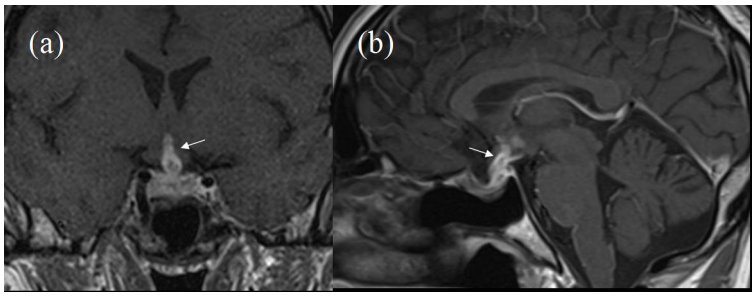


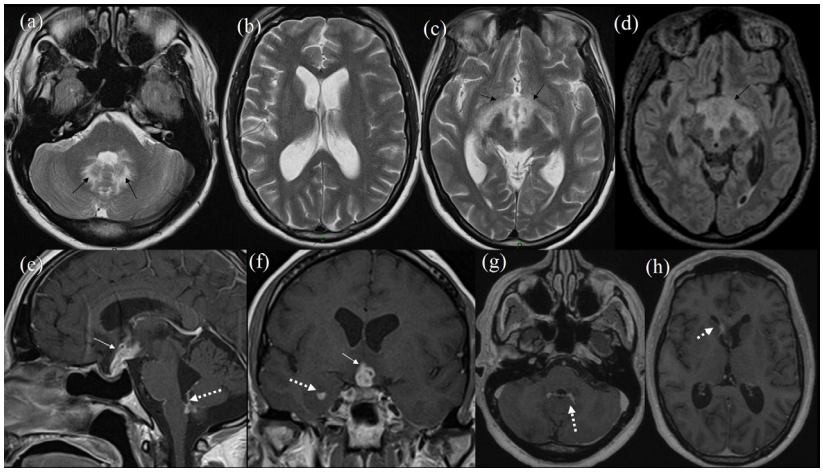

**Figure 2:** Initial MRI During First follow-up. Post-Contrast MRI (a) Coronal and (b) Sagittal Showing some Enhancement along Pituitary Stalk Reaching upto Hypothalamic Region (White Arrows).




changes/oedema was noted in the vicinity of 3rd ventricle, optic tracts, posterior limb of internal capsule bilaterally and anterior aspect of the midbrain. There was further enhancement within the 4th ventricle on both sides and associated prominent oedema in the cerebellum. In addition there was ependymal enhancement within the right frontal and temporal horns (Figure 3). The ventricular size had increased since the previous scan. This rapid growth rate (over two months) and ependymal enhancement was considered unlikely to represent pituitary tumour recurrence and the likelihood of an inflammatory aetiology or lymphoma was considered. He was given dexamethasone 8 mg twice daily which immediately reversed his confusion. At this stage the differential included an inflammatory or lymphomatous process. He became hypothyroid.

The following blood tests were either normal or negative: plasma viscosity, C-reactive protein (CRP), C3, C4, anti-DNA and extractable nuclear antigen (ENA) antibodies.

**Figure 3:** Follow-up MRI. T2 Axial Images (a-c) and FLAIR Axial (d). Black Arrow in (a) Showing Oedema in Cerebellum Around 4th Ventricle. Ventricular Size has Increased Since Previous Scan. Black Arrows in (c, d) Showing Oedema Along Optic Tracts, Posterior Limb of External Capule Extending Posteriorly in Midbrain. Post-Contrast T1 Weighted Sagittal (e), Coronal (f) and Axials (g, h). White Arrow in (e, f) Showing Increase in Enhancing Soft Tissue along Pituitary Stalk and Hypothalamus. Broken White Arrow in (e, g) Showing Ependymal Enhancement in 4th Ventricle. Broken White Arrow in (f) Showing Ependymal Enhancement in Temporal Horn. Broken White Arrow in (h) Showing Ependymal Enhancement in Right Frontal Horn.



NEURO
Open Journal

Openventio
PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-126

Spinal fluid analysis revealed a lymphocytosis of $345\times10^6$/L and a protein of 2459 mg/L. Oligoclonal bands were detected in the cerebrospinal fluid (CSF) only. Cytology and microbiology was negative.

Repeat MRI scan 11 days later showed persistence of an abnormally enhancing tissue in the hypothalamus and pituitary stalk but reduced in volume with some residual enhancement in the 4th Ventricle. The midbrain oedema had resolved when compared to the previous scan. Clinically, he improved but had residual mild impairment of gait. Dexamethasone was reduced and he was discharged on a tapering dose of oral prednisolone.

However, three months later, he was readmitted with worsening confusion. CT head revealed acute hydrocephalus, with increased dilation of the lateral, 3rd and 4th ventricles, and generalised sulcal effacement, with no midline shift. A brain MRI scan confirmed lateral, 3rd and 4th ventricular dilation. Though the abnormally enhancing tissue in the pituitary stalk and hypothalamus had slightly reduced in volume, there was obstruction at the level of 4th ventricle, probably secondary to enhancing lesions with likely adhesions (Figure 4).

He underwent an endoscopic third ventriculostomy and biopsy of the floor of the 3rd ventricle inflammatory lesions.

### Histopathology

Histologically there was abnormal tissue with mixed inflammatory cell infiltrate composed of small lymphocytes, macrophages and plasma cells. There were also occasionally scattered larger cells with large nuclei, prominent nucleoli and abundant eosinophilic cytoplasm. Histopathlogical analysis also showed many CD20-positive B-cells, CD3-positive T-cells, CD138-positive plasma cells as well as CD68-positive macrophages, all confirming the mixed inflammatory cell infiltrate identified on H&E staining. Markers of Langerhan's cell histiocytosis (Langerhans, CD1a) were negative. The conclusion was an acute on chronic reactive process of unknown aetiology. There was no evidence of any neoplasia, including lymphoma or pituitary adenoma. Microbiology specimens were also sent of tissue and CSF, which were all negative. Prolonged culture was performed and there was no evidence of tuberculous disease. Similarly, testing for sarcoid, human immunodeficiency virus (HIV) and syphilis was negative.

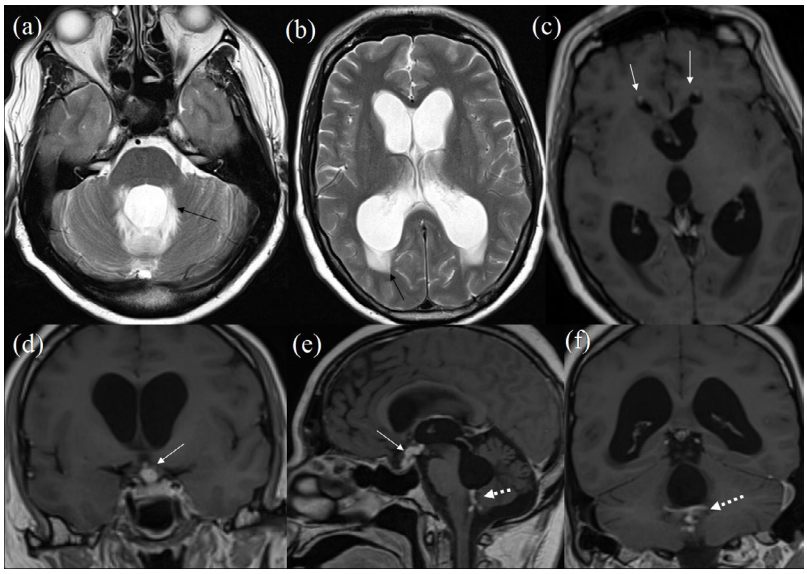
He had insertion of an external ventricular drain, which was converted to a right frontal ventriculo-peritoneal shunt. He was discharged home (on a course of hydrocortisone) and was due to be followed-up for a repeat MRI. Unfortunately, the patient died two months later. The post-mortem identified no cause of death, though it was noted that blood alcohol levels were slightly elevated and it was postulated that he may have failed to self administer oral hydrocortisone.

## DISCUSSION

The current case is complicated by presence of a previously excised benign pituitary adenoma, confirmed on re-analysis of the original histology. The adenoma appeared to have been adequately removed as the initial post-trauma CT confirmed that no definite mass was present.

The patient's presentation to us was thought to be due

**Figure 4:** MRI on Clinical Deterioration. T2 Axials (a, b) Showing Significant Worsening of Hydrocephalus. Black Arrows Showing Significant Periventricular Oedema. Post-Contrast T1 Axial (c), Coronal (d, f) and Sagittal (e). White Arrows in (c) Showing Subtle Residual Enhancement in Frontal Horn. White Arrow in (d, e) Showing Reduced Infundibular-Hypothalamic Enhancement. Broken White Arrows in (e, f) Showing Residual Enhancement on Fourth Ventricular Outflow which Appears Obstructed Causing Hydrocephalus.



Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-126

to an inflammatory process predominantly affecting pituitary-hypothalamic axis with further ependymal involvement at several places that subsequently resulted in obstructive hydrocephalus.

The differential diagnostic possibilities here include infection, inflammation and neoplasia.

Regarding infection; tuberculosis (TB) was considered but culture and biopsies were negative. Radiologically the features of langerhans cell histiocytosis (LCH) were initially thought consistent. Other differential diagnoses include lymphocytic hypophysitis (LYH), sarcoidosis and Erdheim-Chester Disease. The latter which would correlate with the presence of high lymphocytes in the CSF and response to steroids. Regarding sarcoidosis this was contemplated and indeed the CSF findings could be compatible. In sarcoidosis there is often a lymphocytosis with high CSF protein. Oligoclonal banding may also be present. We did not however demonstrate non-caesiating granuloma on histology. Acute disseminated encephalomyelitis (ADEM) and multiple sclerosis were given a thought though dismissed since imaging characteristics did not support this diagnosis. Similarly, there was no histological features of vasculitis seen.

Before histology, a serious consideration was given to LCH. Intra-cranial LCH lesions exist in two different forms: hypothalamic-pituitary axis change (HPA-LCH), being the most common, and less frequently neurodegenerative change (ND-LCH). In HPA involvement, central diabetes insipidus (CDI) arises in up to 50% of cases,[1] and anterior pituitary hormone deficiencies in 20%.[2] CDI is rare and characterised by polyuria, polydipsia and hypotonic urine due to antidiuretic hormone deficiency and, whilst LCH is a cause, it is by no means the most common cause.[3,4] Conversely, the latter pattern presents with a mixture of ataxia, nystagmus, dysarthria, spastic tetraparesis, pseudobulbar palsy or cognitive/behavioural alterations[5] – the first two and latter symptoms were seen in our patient but not CDI. HPA-LCH presents on MRI with a thickened hypothalamus, thickened pituitary stalk and absent posterior pituitary bright spot. In patients presenting with diabetes insipidus.[6,7] ND-LCH affects the cerebellum, basal ganglia and pons. MRI can show bilateral signal changes and non-enhancing lesions in the dentate nucleus, often extending to the surrounding white matter involving the midbrain, pons, globus pallidus and cerebellar peduncle or presenting with cerebellar atrophy.[8-12]

There are varying opinions on the indication for HPA biopsy in terms of the size of the lesion or severity of symptoms.[13] Studies by Jian et al[13], Leger et al[14] and Adani et al[15] all propose different cut-offs for biopsy, such as presence of an endocrinopathy, radiological changes (e.g., when the stalk is >7 mm) and/or clinical deterioration.

In the past, electron microscopy was used on these biopsy specimens to detect the Birbeck granules that were characteristic of the LC; however this has been replaced by the use of immunohistochemistry to detect CD207 (Langerin) or CD1a expression. CD207 is a type II transmembrane C-type lectin associated with the formation of Birbeck granules.[16] This test is now the Gold Standard in the correct clinical setting.[17,18]

Erdheim-chester disease (ECD) is a rare disease, frequently seen in adults. It shows infiltration of a wide variety of tissues by cells of macrophage and histiocytic lineage, considered distinct from LCH. It is an infiltrative process with preponderance of lipid-laden histiocytes mixed with lymphocytes and eosinophils. It can vary from a focal abnormality to an extensive multi-organ and life-threatening condition. It can show endocrine manifestations including CDI, as well as hypopituitarism and hyperprolactinaemia. However, on MRI, pituitary mass lesions or stalk thickening is rarely found although normal T1 high signal is frequently absent from the posterior pituitary. ECD was considered but excluded due to lack of typical findings and lack of systemic involvement.[19] LYH or autoimmune hypophystis is characterised by focal or diffuse inflammatory infiltration and variable degree of pituitary involvement.[19] It can be divided into three subtypes: lymphocytic adenohypophysitis, lymphocytic infundibuloneurohypophysitis and lymphocytic panhypophysitis. It can be frequently associated with other autoimmune conditions, most commonly autoimmune thyroid disease (reported in 15-25% of LYH cases, 75% of these being chronic autoimmune thyroiditis). Autoantibodies to various cells can be found, including prolactin, GH and Adrenocorticotropic hormone (ACTH) secreting cells. In our case, due to previous pituitary surgery, assessment of these was not thought to be of use. Histopathology is the gold standard for diagnosis of LYH, the characteristic feature being a diffuse polyclonal lymphocytic infiltrate with predominance of T-cells, particularly CD4 cells. Clinically, it can present with headache, impaired vision, nausea, vomiting, fatigue, weakness, anorexia, and visual disturbances. There can be partial or total hypopituitarism.[19]

Sarcoidosis is a multisystem granulomatous disorder.[20] Neurosarcoidosis occurs in about 10% of affected patients. The disease primarily involves leptomeninges and extends along perivascular spaces resulting in parenchymal lesions. The lesions have predilection for basal structures of brain, including hypothalamus and pituitary gland. Therefore involvement of hypothalamic-pituitary axis (resulting in CDI and anterior pituitary failure) is the most common feature of neurosarcoidosis. On MRI, the lesions are seen as infiltration of the dura mater which is thickened and show enhancement on contrast, also involving infundibular stalk, optic chiasm and floor of third ventricle that can be quite thickened, sometimes with mass like areas.[20]

CNS lymphoma is another entity that can be primary or secondary. It could clearly have been a case of either of these, although primary more likely due to absence of systemic disease. These can be steroid responsive initially. However, histology was not supportive. On reflection, amongst all possibilities, the imaging findings were considered closest to LCH although disproved on histolopathology. The histology itself was more in

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-126

favour of a LYH but the inflammatory deposits seen on MRI imaging were too extensive for this condition in isolation. Therefore, there was absence of clear similarity with any of the known conditions affecting this region, leading us to conclude that this was a case of steroid responsive inflammatory process of unknown aetiology.

The patient was treated with steroids and subsequent MRI did prove that there was improvement in hypothalamic/infundibular lesion. However, the ependymal involvement was associated with adhesions that led to 4th ventricle outflow obstruction and acute obstructive hydrocephalus with clinical deterioration. While this was noted earlier, this continued to progress despite the use of steroids. This clearly shows that depending upon the site of involvement, a steroid responsive inflammatory condition can still have adverse outcome. Those with involvement of the fourth ventricle and potentially the sylvian aqueduct should be closely followed with early imaging, to detect hydrocephalus.

It is worth noting that this patient did not die of acute hydrocephalus and large inflammatory lesions within the brain were not seen on post-mortem examination. The cause of death is unknown, but not thought to be directly related to the disease process in question.

## CONCLUSION

We present an unusual case, complicated by previous surgery, but clearly inflammatory in origin predominantly affecting the suprasellar region but extending elsewhere to involve ependymal lining. This case highlights the fact that despite extensive literature and rather well addressed reviews available, it is possible to have a rare inflammatory condition, that can mimic other inflammatory conditions such as LCH or LYH in different ways, yet probably represents another entity not yet fully characterised. It also highlights that despite the steroid responsive nature, the ependymal involvement can result in progressive acute obstructive hydrocephalus with clinical deterioration, thereby suggesting close follow-up and early imaging for early detection and treatment of this complication.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of intertest.

## CONSENT

Written informed consent was obtained from the patient for publication of this case report and any images.

## REFERENCES

1. Gallo MG, Martínez MP, Abalovich MS, Gutiérrez S, Guitelman MA. Endocrine manifestations of Langerhans cell histiocytosis diagnosed in adults. *Pituitary*. 2010; 13(4): 298-303. doi: 10.1007/s11102-010-0233-8

2. Kaltsas G.A, Powles TB, Evanson J, et al. Hypothalamo-pituitary abnormalities in adult patients with langerhans cell histiocytosis: Clinical, endocrinological, and radiological features and response to treatment. *J Clin Endocrinol Metab*. 2000; 85(4): 1370-1376. doi: 10.1210/jcem.85.4.6501

3. Mootha SL, Barkovich AJ, Grumbach MM, et al. Idiopathic hypothalamic diabetes insipidus, pituitary stalk thickening, and the occult intracranial germinoma in children and adolescents. *J Clin Endocrinol Metab*. 1997; 82(5): 1362-1367. doi: 10.1210/jcem.82.5.3955

4. Wang LC, Cohen ME, Duffner PK. Etiologies of central diabetes insipidus in children. *Pediatr Neurol*. 1994; 11(4): 273-277. doi: 10.1016/0887-8994(94)90001-9

5. Mimouni-Bloch A, Schneider C, Politi KE, et al. Neuropsychiatric manifestations in Langerhans' cell histiocytosis disease: A case report and review of the literature. *J Child Neurol*. 2010; 25(7): 884-887. doi: 10.1177/0883073809351317

6. Mendoza ES, Lopez AA, Valdez VAU, Uy-Ho JD, Kho SA. Adult-onset Langerhans cell histiocytosis presenting with adipsic diabetes insipidus, diabetes mellitus and hypopituitarism: A case report and review of literature. *J Clin Transl Endocrinol*. 2015; 1(1): 1-5. doi: 10.1016/j.jecr.2015.06.001

7. Maghnie M, Cosi G, Genovese E, et al. Central diabetes insipidus in children and young adults. *N Engl J Med*. 2000; 343(14): 998-1007. doi: 10.1056/NEJM200010053431403

8. Grois N, Fahrner B, Arceci RJ, et al. Central nervous system disease in Langerhans cell histiocytosis. *J Pediatr*. 2010: 156(6); 873-881. doi: 10.1016/j.jpeds.2010.03.001

9. Prayer D, Grois N, Prosch H, Gadner H, Barkovich AJ. MR imaging presentation of intracranial disease associated with Langerhans cell histiocytosis. *AJNR Am J Neuroradiol*. 2004; 25(5): 880-891.

10. Martin-Duverneuil N, Idbaih A, Hoang-Xuan K, et al. Langerhans Cell Histiocytosis Study Group. MRI features of neurodegenerative Langerhans cell histiocytosis. *Eur Radiol*. 2006; 16(9): 2074-2082. doi: 10.1007/s00330-006-0200-y

11. Ertan G, Huisman TA. Susceptibility-weighted imaging in neurodegeneration in Langerhans cell histiocytosis. *J Pediatr*. 2010: 156(6); 1032. doi: 10.1016/j.jpeds.2009.11.081

12. Spagnolo F, Leopizzi E, Cardamone R, et al. Neurodegeneration in the course of Langerhans cell histiocytosis. *Neurological Sciences*. 2012:33(3); 605-607. doi: 10.1007/s10072-011-0677-2

13. Jian F, Bian L, Sun S, et al. Surgical biopsies in patients with central diabetes insipidus and thickened pituitary stalks. *Endo-*

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-126

*crine*. 2014:47(1); 325-335. doi: 10.1007/s12020-014-0184-3

14. Leger J, Velasquez A, Garel C, Hassan MAX, Czernichow P. Thickened pituitary stalk on magnetic resonance imaging in children with central diabetes insipidus 1. *J Clin Endocrinol Metab*. 1999; 84(6): 1954-1960. doi: 10.1210/jcem.84.6.5745

15. Beni-Adani L, Sainte-Rose C, Zerah M, et al. Surgical implications of the thickened pituitary stalk accompanied by central diabetes insipidus. *J Neurosurg*. 2005: 103(Suppl 2); 142-147. doi: 10.3171/ped.2005.103.2.0142

16. Emile JF, Abla O, Fraitag S, et al. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. *Blood*. 2016; 127(22): 2672-2681. doi: 10.1182/blood-2016-01-690636

17. Nakamine H, Yamakawa M, Yoshino T, Fukumoto T, Enomoto Y, Matsumura I. Langerhans cell histiocytosis and Langerhans cell sarcoma: Current understanding and differential diagnosis. *J Clin Exp Hematop*. 2016; 56(2): 109-118. doi: 10.3960/jslrt.56.109

18. Jaffe R. The diagnostic histopathology of Langerhans cell histiocytosis. In: Weitzman S, Egeler RM, eds. *Histiocytic Disorders of Children and Adults*. Cambridge, MA, USA: Cambridge University Press; 2005: 14-39.

19. Carpinteri R, Patelli I, Casanueva F, Giustina A. Inflamamtory and granulomatous expansive lesions of the pituitary. *Best Pract Res Clin Endocrinol Metab*. 2009; 23(5): 639-650. doi: 10.1016/j.beem.2009.05.009

20. Saleem SN, Sai AHM, Lee DH. Lesions of the hypothalamus: MR imaging diagnostic features. *Radio Graphics*. 2007; 27: 1087-1108. doi: 10.1148/rg.274065123

NEURO

Openventio PUBLISHERS

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-127

Research

*Corresponding author
**Avinash Kumar Kanodia**, MD, DM, FRCR
Department of Radiology
Ninewells Hospital and Medical School
Dundee, UK
**E-mail:** avinash.kanodia@nhs.net

*Volume 4 : Issue 1*
*Article Ref. #: 1000NOJ4127*

***Article History***
**Received:** July 2nd, 2017
**Accepted:** August 29th, 2017
**Published:** August 29th, 2017

***Citation***
Badran K, Abdelsadq M, Kouli O, et al. Audit of a standard operating procedure to prevent wrong-level lumbar spinal surgery with intra-operative X-ray. *Neuro Open J*. 2017; 4(1): 31-37. doi: 10.17140/NOJ-4-127



# Audit of a Standard Operating Procedure to Prevent Wrong-Level Lumbar Spinal Surgery with Intra-Operative X-Ray

**Khaled Badran, MBBS, MRCS (Ed)[1]; Mohamed Abdelsadq, MBBS, PG.Dip[1]; Omar Kouli, Medical Student[1]; Avinash Kumar Kanodia, MD, DM, FRCR[2*]; Eric Ballantyne, MBChB, MD, FRCS[1]; Kismet Hossain-Ibrahim, MBChB, FRCS, PhD[1]; James Galea, MBChB, FRCS, PhD[1]; David Mowle, MBChB, FRCS, MD[1]**

*[1]Department of Neurosurgery, Ninewells Hospital and Medical School, Dundee, UK*
*[2]Department of Radiology, Ninewells Hospital and Medical School, Dundee, UK*

**ABSTRACT**

**Introduction:** One of the major errors that can be encountered by a spinal surgeon is operating at the wrong level/side. However, wrong-level spinal surgery is considered a 'never-event' and is under-reported. Many surgeons have traditionally adopted the technique of palpating or "counting" from L5-S1 to determine the operative level in lumbar spine procedures without necessarily the use of intraoperative X-ray control. Most surgeons these days; however, use X-rays or fluoroscopy during the surgery. There is no universal standard operating procedure (SOP) for the use of X-rays or fluoroscopy during spinal surgery and the compliance of the surgeons for any local SOP is unknown.
**Aim:** The audit primarily intended to check the compliance with an established local SOP using X-ray to identify lumbar spinal level. We also determined the accuracy of lumbar spine level marking by palpation. We also tried to quantify the intra-operative error rate following pre-operative X-ray level marking. Overall, the optimum role of X-rays was determined for adequate level of lumber decompression.
**Methods:** The audit was performed as a prospective clinical audit within a single neurosurgical department. Data collected from theatre logbook, medical notes and picture archive and communication system (PACS). An established local SOP for use of X-rays during spinal surgery was used as a benchmark to audit local practice.
**Cycle 1:** Every lumbar discectomy and decompression from June to November 2015 (6 months) was obtained. The findings were presented in our local clinical effectiveness meeting with the aim check local practice and suggest improvements.
**Cycle 2:** Re-audit a further 6 months, December 2015 to May 2016, to see the significance of the change implemented.
**Results:** In the first cycle, one patient did not receive pre-operative X-ray. While all other patients received pre-operative X-rays, the number of exposures was available in only 71% of patients, out of which 39% required one exposure, 43% required two exposures, 16% required three exposures and 2% required four exposures. Twenty eight cases (13.9%) were recorded to have intra-operative X-ray level checked due to doubt, out of which 22 cases were found to be on an incorrect level.
In the second cycle, all patients received pre-operative X-rays and the number of exposures was recorded for all, out of which 52% required one exposure, 32% required two exposures, 13% required three exposures and 3% required four exposures. Twenty cases (9.7%) were recorded to have intra-operative X-ray level checked due to an arising doubt, out of which only 7 were found to be on an incorrect level.

**KEY WORDS:** Wrong level surgery; Pre-operative X-rays; Intra-operative X-rays.

**ABBREVIATIONS:** SOP: Standard Operating Procedure; PACS: Picture Archive and Communication System; NHS: National Health Service.

NEURO
Open Journal

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-127

## INTRODUCTION

One of the most preventable errors that could occur during spinal surgery is operating on the wrong site. Surgery done on the wrong site includes either operating at an unplanned side or level. In spinal surgery, the most common error for wrong site surgery is a procedure done one level above the intended level.[1]

Wrong-level spinal surgery is considered a 'never-event' and is under-reported.[2] The rate of wrong level spine surgery ranges widely in the literature. It is estimated that at least 50% of spine surgeons will perform at least one wrong level procedure in their career.[3,4] A national survey done on incorrect site surgery among neurosurgeons in Canada-based on anonymous questions self-assessment-concluded that corrected wrong level lumbar discectomies rate was estimated to be 12.8 per 10,000 operations, which was much higher than cervical discectomies at 7.6 per 10,000 operations. Risk factors for such occurrences were recognized to be fatigue, increased time pressure and urgent operations.[5]

A wrong level surgery can potentially have significant emotional, physical, financial and legal consequences on the patient as well as surgeons and the importance of preventing this event cannot be overemphasized.[1]

Careful pre-operative assessment along with robust pre- and intra-operative localization techniques is crucial in improving the quality of the surgery. Historically, the surgeons use palpation or "counting" from a fixed reference point such as L5-S1 vertebrae for determining the level of operation in lumbar spine surgery.[6] More recently, X-rays and fluoroscopy are increasingly and almost universally used. However, use of X-rays once and only at the beginning of the procedure is not thought to be enough and there are standard operating procedures (SOPs) occasionally suggested to define the use of X-rays during these procedures,[5] which are sometimes modified by organizations for local use. It remains the collective responsibility of the organization; however, to ensure that such SOPs are strictly followed to avoid errors during the surgery. We could not find any such audit in the literature regarding compliance with similar SOPs elsewhere. We performed a prospective audit in our department to assess if the SOP was being followed.

## METHODS AND TECHNIQUE

This is a prospective audit of a total of 409 patients undergoing a lumbar discectomy or decompression within a single neurosurgical department. Caldicott approval has been taken for this audit.

The SOP followed in our department has been described below:

- After general anesthesia, patients lies in prone position
- Check list and consent confirmed out loud with theater team
- Skin palpation of spinous process for counting level
- 18 G needle inserted vertically through skin at a disc space level
- Lateral X-rays obtained using Siemens Ziehm vision (Small field of view of 0.8 m²).

1. Step 1 X-ray: Pre-operative X-ray to identify the targeted level, which should be confirmed by 2 clinicians (compulsory). It is considered adequate if the needle-points towards disc space;

2. Step 2 X-ray: Intra-operative X-ray after skin incision but before decompression in case of doubt of the trajectory (optional);

3. Step 3 X-ray: Intra-operative X-ray at the end of the procedure and prior to closure, either marking the inside of the disc space or the superior and inferior edges of the decompression (compulsory).

The number of X-ray exposures should be recorded for each step. This SOP has been adopted from Tayside National Health Service (NHS) healthcare and the broad information available from NHS choice UK[5] as there are no general universal SOP descried in literature particularly in the US or the Royal Colleges. We have modified the local SOP by adding an intra-operative X-ray.

In view of the clinical importance of following the SOP during surgery, in one of the morbidity and mortality meetings in Department of Neurosurgery in 2015, it was decided to perform a prospective audit in the department to check compliance to the SOP. It was meant to be a clinical audit rather than a research project and in accordance with local practice, the decision of the department was felt to be adequate in carrying out the audit project.

The first cycle of this audit included 202 patients from June to November 2015 (6 months).

The second cycle (re-audit) included 207 patients from December 2015 to May 2016, to see the significance of the change implemented. The data was collected from theatre logbook, medical notes and PACS. Number of times intra-operative X-rays detected an incorrect level at each stage of the procedure was identified. Categorical factors (level of pathology and correct/incorrect level in interpretative level check) were tested with Chi-squared test. Continuous factors (number of pre-operative X-rays required) were tested using the Mann-Whitney U-test. $p$ value were calculated for both with $p<0.05$ as significant.

## RESULTS

### In the First Cycle

There were 202 patients included (114 had a discectomy and 88 had decompression). One patient did not have pre-operative X-ray step 1 level check; however, the number of step 1 X-ray exposures was recorded in only 143 cases. The number of pre-operative step 1 X-rays required for those patients were as follows: 39% required one exposure, 43% required two exposures,

NEURO
Open Journal

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-127

16% required three exposures and 2% required four exposures (Figure 1). Palpation was found to be accurate in 39% patients.

Intra-operatively step 2 X-ray (optional) was performed in 28 cases (13.9%); 8 cases (26.6%) confirming the correct level; however, in 18 cases (66.8%) the X-ray detected a wrong level before the decompression, nonetheless in 2 cases (6.6%) the step 2 X-ray was obtained after the fenestration or decompression (Figure 2). Every patient had X-ray step 3 level check before closure.

**In the Second Cycle (Re-Audit)**

There were 207 patients included (127 had a discectomy and 80 had decompression). All patients in the second cycle had a pre-operative X-ray step 1 check. The number of pre-operative X-rays required for those patients were as follows: 52% required one exposure, 32% required two exposures, 13% required three exposures and 3% required four exposures (Figure 1). Palpation was found to be accurate in 52% patients.

Intra-operatively step 2 X-ray was performed in 22 cases (10.6%); 13 cases (59.1%) confirmed the correct level, 7 cases (31.8%) were on the incorrect level before decompression and 2 of the cases (9.1%) had incorrect level after fenestration and decompression (Figure 2). Every patient had X-ray step 3 level check before closure.

We also decided to look at the number of patients who required further decompression during their surgery in the re-audit. We obtained a step 3 X-ray level check of the superior and inferior edges of lumber decompression and checked for adequate decompression. We found that 8 patients out of 80 required further decompression superior or inferior as it was deemed inadequate by the surgeon. 2 cases were single level, 2 cases were 2 levels and 4 cases involved three or more levels of

**Figure 1:** Comparative Graph of the Number of Pre-operative X-ray (Step 1) Needed in both Cycles.

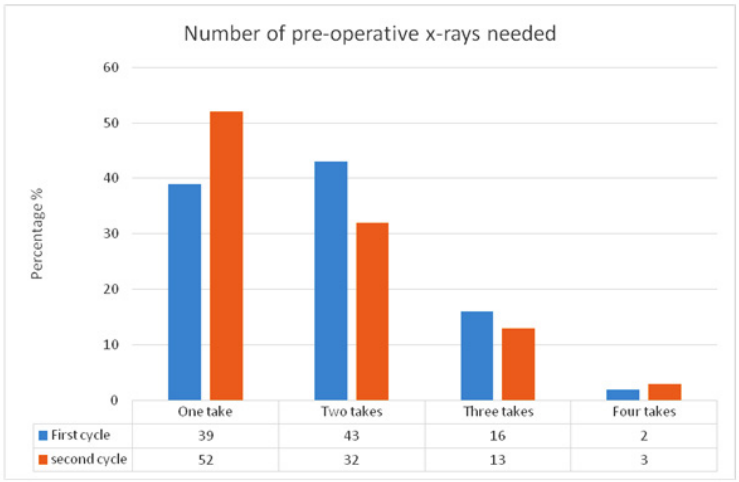
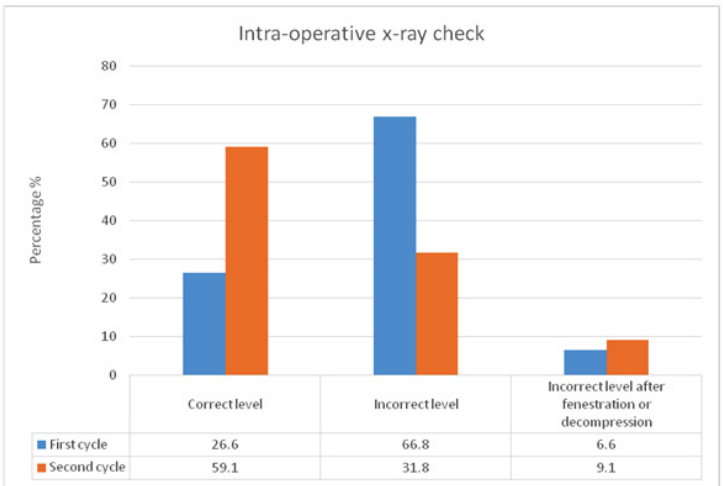



**Figure 2:** Percentage of Correct and Incorrect Levels During Intra-operative X-ray Check (Step 2) when in Doubt in both Cycles.

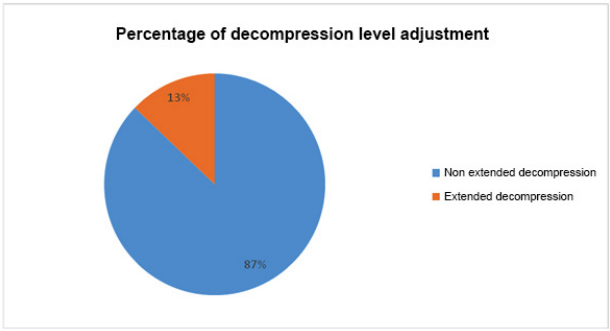


NEURO
Open Journal

Openventio
PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-127

**Figure 3:** Percentage of Patients having Extended Decompression in Second Cycle (Re-Audit).




decompression (Figure 3).

The most common level of pathology identified in the second cycle was L4-L5 followed by L5-S1. There was a statistically significant correlation ($p$=0.0435, Chi square test) between the level of pathology and the use of intra-operative X-ray check in cases of doubt (Figure 4). However, there was no significant correlation between the pathology level and localization errors ($p$>0.05).

There was no difference in surgical time before and after this audit (first and second cycles).

Notably, transitional vertebra did not have any implication of the wrong level marking or the surgical counting because it was well agreed prior to surgery between the 2 clinicians on what to call each level and how to determine the right level on X-ray (Figures 5, 6, 7 and 8).

**Figure 4:** Level of Pathology and the Percentage of Intra-operative X-ray Check (Step 2) Needed in Cases of Arising Doubt in Each Intervertebral Level ($p$<0.05).

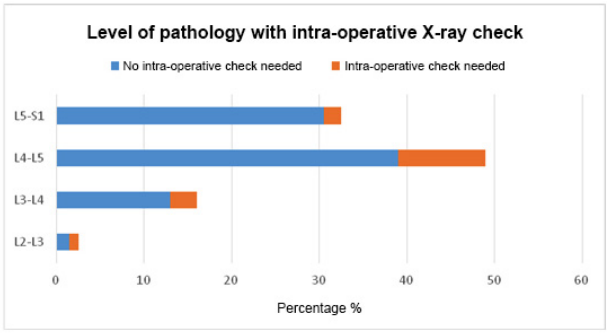
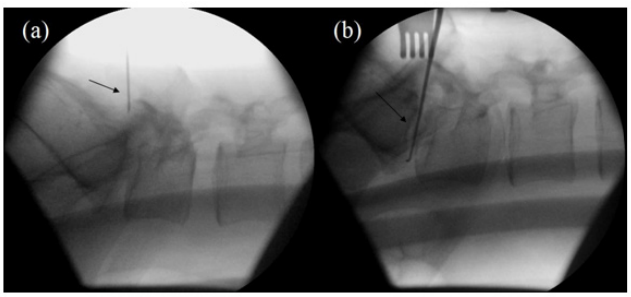



**Figure 5:** Pre-operative (Step 1) X-ray (a) Shows Needle (Arrow) Projecting towards L5/S1 disc. Intra-operative X-ray (Step 3) in (b) Showing Hook (arrow) within L5/S1 Disc Space.



NEURO
Open Journal

Openventio
PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-127

**Figure 6:** Pre-operative (Step 1) X-ray (a) Shows Needle (Arrow) Projecting Towards L4/5 disc. Intra-operative (Step 2) X-ray (b) Shows Needle (arrow) below L4/5 disc. Intra-operative X-ray (Step 3) (c) Shows the Hook (arrow) within L4/5 disc.

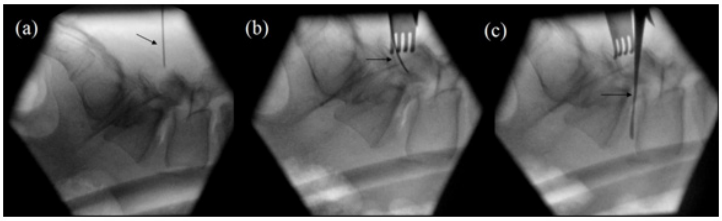



**Figure 7:** Intra-operative (Step 3) X-ray Shows Hooks (Arrows) at the Superior and Inferior Margins of Decompression.

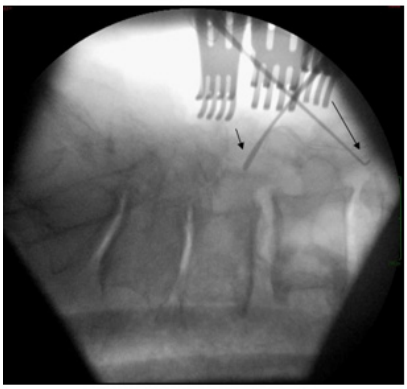
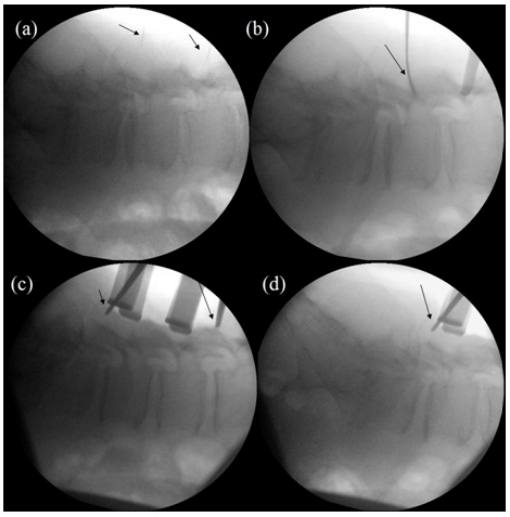


**Figure 8:** Preoperative (Step 1) X-ray (a) Shows Needles (Arrows) at Superior and Inferior Margins of Planned Multilevel Decompression. Intra-operative (Step 2) X-ray (b), Confirms the Level by the Needle (Arrow). Intra-operative (Step 3) X-ray (c) Shows Hooks (Arrows) at the Superior and Inferior Margins of Decompression, However, the L5/S1 Junction is Not Included. Intra-operative (Step 3) X-ray (d) Shows Hook (Arrow) at the Inferior Margins of Decompression, the L5/S1 Junction is Now Included, thereby Confirming the Exact Level of Decompression.




## DISCUSSION

Spinal surgery is a complex procedure, which involves a coordinated team work and significant planning. Appropriate communication is crucial element to ensure the best outcome and to avoid any unnecessary complication or errors such as wrong level surgery because of the significant implications for the patient, the surgical team and the institution.

Even though wrong level spinal surgery is a "never event" however it is still one of the potential complications and suspected to be under reported.[1] A national survey conducted by Mayer et al identified that 55% of surgeons disclosed that they started operating on a wrong spinal level, which was corrected intra-operatively.[7]

To ascertain the correct level of surgery, traditionally, the most common anatomical landmark for palpation has been counting the facet joint and spinous process followed by lamina and interspinous ligaments.[2] There are several potential reasons why it may be difficult to consistently and accurately establish correct level by palpation. One in five patients do not have the normal fifth lumbar-type vertebra. Patients with unusual spine anatomy or deformity such as scoliosis or hyperlordotic make it difficult to count the correct level. Use of intra-operative imaging on morbidly obese patients is also challenging and has a higher risk of errors.[8] It was notable that the palpation alone was found to be accurate only in 39% and 52% patients in cycles 1 and 2 respectively.

The use of X-rays to localize the level has emerged and established as a very important method to prevent the risk of wrong level surgery.[1] However, there is no consensus about optimum use of X-rays during the surgery. The use of X-rays during

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-127

surgery can vary from pre-operative X-rays to various combinations of intra-operative X-rays or fluoroscopy. Both Spot X-rays and fluoroscopy have been used by spinal surgeons without clear preference. A national survey of members of the North American Spine Society (NASS) showed a 56% reported using plain radiographs and 44% used fluoroscopy as the localization method.[9] Some of the survey results would suggest that a substantial number of surgeons use both techniques in combination.[7]

Use of pre-operative X-rays alone, although thought to be widely in use, may be inadequate. Performing lumbar microsurgery involves a very small incision. In fact, one level lumbar spine incision in a microdiscectomy or microdecompression is approximately 2.5-4 cm. This gives a very small window to identify the appropriate operating level.[6] In our local SOPs, therefore, we include additional (Step 2 and Step 3 X-ray exposures) to confirm that a correct and adequate level is being operated upon.

The aim of this audit was to review our compliance with our SOPs using multi step X-rays to identify lumbar spinal level, determine the accuracy of lumbar spine level marking by palpation, the intra-operative error rate following pre-operative X-ray level marking and to determine the adequacy of decompression during lumbar spine surgery. No Step 1 pre-operative X-rays were obtained for 1 patient in cycle 1, although 100% compliance was noted in cycle 2. The recording of number of X-ray exposures also clearly improved to 100% during cycle 2. We identified that using anatomical landmarks as a localization method was often difficult with 1 exposure alone and as a result we had to take more than single exposure (step 1) in at least 48% of our patients. We also found that using additional X-rays (step 2 and step 3) gave us the opportunity to correct the surgical level during surgery and prevented wrong level surgery in 27 cases. In 8 cases we found that the operating surgeon deemed the decompression level was insufficient after (step 3) X-ray of the superior and inferior margin and further extension of surgery was required. Most of the cases needed superior margin extension of decompression. The risk was also higher in multilevel decompression rather than single level. Eventually, at the end of procedure, all our patients had adequate surgery performed in both cycles.

It was also noticed that the most common level of pathology was L4-L5 level, which was also associated with a significantly higher incidence ($p<0.05$) of intra-operative doubt resulting in use of additional X-rays exposures to identify any localization errors.

The current audit clearly shows palpation and even pre-operative X-rays as being inadequate in ensuring correct level surgery or adequate decompression. It establishes the superiority of our SOP by using X-rays during surgery at different stages rather than merely at the beginning of the procedure. We are not aware if this practice is widespread or limited as no published data is available regarding the same; our audit is the first known audit of its kind. Also, as we noticed, it is also important to continue to audit practice so ensure that the SOP is also followed in real practice including all steps, as bypassing some of the steps can potentially result in wrong level surgery with its associated complications.

## CONCLUSION

We have developed an SOP in our department that essentially involves multi-step X-rays during lumbar spinal surgery to accurately identify and then reconfirm correct level during the procedure that allows necessary corrections at appropriate time. We have also highlighted that use of pre-operative X-ray alone is inadequate. We have also audited our practice to ensure that the SOP is followed appropriately in our institution. Our results show that it is possible to avoid both, a wrong level spinal surgery for discectomy and ensuring adequate surgery for decompression (including multilevel), by developing and adopting such SOP.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Goodkin R, Laska LL. Wrong disc space level surgery: Medicolegal implications. *Surg Neurol*. 2004, 30; 61(4): 323-341. doi: 10.1016/j.surneu.2003.08.022

2. Jhawar BS, Mitsis D, Duggal N. Wrong-sided and wrong-level neurosurgery: A national survey. *J Neurosurg Spine*. 2007; 7(5): 467-472. doi: 10.3171/SPI-07/11/467

3. Wong DA, Watters WC. To err is human: Quality and safety issues in spine care. *Spine (Phila Pa 1976)*. 2007; 32(Suppl 11): S2-S8. doi: 10.1097/BRS.0b013e318053d4cd

4. Mody MG, Nourbakhsh A, Stahl DL, Gibbs M, Alfawareh M, Garges KJ. The prevalence of wrong level surgery among spine surgeons. *Spine*. 2008, 33(4): 194-198. doi: 10.1097/BRS.0b013e31816043d1

5. National Health Service (NHS). Lumbar decompression surgery - How it's performed. Web site. www.nhs.uk/Conditions/Lumbardecompressivesurgery/Pages/Surgery.aspx. Accessed July 1st, 2017.

6. Irace C. Intraoperative imaging for verification of the correct level during spinal surgery. In: Fountas Kn, ed. *Novel Frontiers of Advanced Neuroimaging*. Rijeka, Croatia: INTECH Open Access Publisher; 2013: 174-188. doi: 10.5772/52055

7. Mayer JE, Dang RP, Prieto GF, Cho SK, Qureshi SA, Hecht AC. Analysis of the techniques for thoracic-and lumbar-level localization during posterior spine surgery and the occurrence of

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-127

wrong-level surgery: Results from a national survey. *Spine J*. 2014; 14(5): 741-748. doi: 10.1016/j.spinee.2013.06.068

8. Hsiang J. Wrong-level surgery: A unique problem in spine surgery. *Surg Neurol Int*. 2011; 2: 47.

9. Irace C, Corona C. How to avoid wrong-level and wrong-side errors in lumbar microdiscectomy. *J Neurosurg Spine*. 2010; 12(6): 660-665. doi: 10.3171/2009.12.SPINE09627

NEURO

Openventio PUBLISHERS

ISSN 2377-1607 Open Journal http://dx.doi.org/10.17140/NOJ-4-128

Research

*Corresponding author
**Rafael Coveñas, MSc, PhD**
Universidad de Salamanca
Instituto de Neurociencias de Castilla y León (INCYL), Laboratorio 14
c/ Pintor Fernando Gallego, 1, 37007 Salamanca, Spain
**Tel.** 34-923-294400; ext 5315
**Fax:** 34-923-294549
**E-mail:** covenas@usal.es; mangasam@usal.es

*Volume 4 : Issue 1*
*Article Ref. #: 1000NOJ4128*

***Article History***
**Received:** July 21st, 2017
**Accepted:** September 12th, 2017
**Published:** September 12th, 2017

***Citation***
Tabera S, Mangas A, Geffard M, Coveñas R. Immunomodulatory effects of mesenchymal stem cells on T- and B-cells in a quiescent state in a chronic experimental model of autoimmune encephalomyelitis. *Neuro Open J*. 2017; 4(1): 38-49. doi: 10.17140/NOJ-4-128



# Immunomodulatory Effects of Mesenchymal Stem Cells on T- and B-Cells in a Quiescent State in a Chronic Experimental Model of Autoimmune Encephalomyelitis

**Soraya Tabera, MSc, PhD[1]; Arturo Mangas, MSc, PhD[1,2]; Michel Geffard, MD, PhD[2,3]; Rafael Coveñas, MSc, PhD[1*]**

*[1]Institute of Neurosciences of Castilla y León (INCYL), Laboratory of Neuroanatomy of the Peptidergic Systems, University of Salamanca, Salamanca, Spain*
*[2]Gemacbio, Research Department, Lieu dit Berganton, Saint Jean d'Illac, France*
*[3]Institut pour le Développement de la Recherche en Pathologie Humaine et Thérapeutique (IDRPHT), Talence, France*

## ABSTRACT

**Introduction:** Multiple sclerosis (MS) is an immune-mediated disease affecting the central nervous system (CNS). Many drugs have been tested in animal models of MS (e.g., Experimental Autoimmune Encephalomyelitis (EAE)). Nevertheless, clinical observations indicate that suppression of the immune response is a very simple approach to address the problem, since the injuries produced by the inflammation do not predict later changes. An emerging strategy for neuroprotection and remyelination is the transplantation of stem cells. Mesenchymal stem cells (MSC) have been characterized by their multipotentiality and their capacity for immunomodulation, thus raising great expectations in regenerative medicine.
**Materials and Methods:** In this context, we have tested the therapeutic potential of intravenously injected bone marrow-MSC from healthy rat donors in a chronic EAE model using Lewis 1A rats. We analyzed the role of MSC on T- and B-cells in the quiescent state.
**Results:** Rat MSC expressed the vascular cell adhesion molecule CD106 to a slight extent. MSC promoted T- or B-lymphocyte survival but did not modify the T- or B-lymphocyte cell cycles in the quiescent state. Our results also confirm that MSC modulate EAE though the production of soluble cytokines. *In vitro,* MSC decreased the EAE by immunomodulating the Th1/Th2 response. Moreover, MSC controlled CD27/CD86 expression in different ways.
**Conclusion:** Animals infused with MSC prior to the EAE immunization did not develop EAE, or their EAE clinical scores were decreased, whereas animals that received MSC after the induction of EAE developed a normal EAE course. This novel therapeutic strategy would further our knowledge of the pathophysiology of autoimmune diseases, which in the future could be translated into clinical application.

**KEY WORDS:** Multiple sclerosis (MS); Lymphocyte; Immunomodulation; MSC; EAE.

**ABBREVIATIONS:** MS: Multiple Sclerosis; EAE: Experimental Autoimmune Encephalomyelitis; MSC: Mesenchymal Stem Cells; MOG: Myelin Oligodendrocyte Glycoprotein; CNS: Central Nervous System.

## INTRODUCTION

Although, there are still many problems to be solved before cell therapy can be used reliably to repair mature central nervous system (CNS) lesions, one strategy that could be used in multiple sclerosis (MS) is the transplantation of stem cells. Several types of stem cells have been studied and characterized: embryonic stem cells, neural stem cells, haematopoietic stem cells, non-haematopoietic stem cells and bone marrow stromal cells.[1-7] In this regard, neural precursor cells

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

were the first candidates for cell-based therapy in neuroinflammation.[8,9] However, the invasiveness associated with harvesting neural precursor cells, together with the reduced number of cells that can be obtained, may limit their clinical application. A potential alternative to neural precursor cells is mesenchymal stem cells (MSC). Recently, the actions exerted by adipose-derived mesenchymal stromal cells,[10-14] placenta-derived mesenchymal stem cells,[15,16] Wharton's jelly mesenchymal stem cells,[17] human decidua-derived mesenchymal stem cells[18] and human umbilical cord blood mesenchymal stromal cells[19] have been reported in experimental autoimmune encephalomyelitis (EAE) animal models.

MSC have been characterized by their multipotentiality and their capacity for immunomodulation, thus raising great hopes in regenerative medicine. These cells could be ideal candidates for application in human diseases, since they offer significant practical advantages: they are obtained from different adult tissues and can readily be cultured and expanded; they are multipotent and self-renewing. They have the capacity to differentiate into mesodermal-derived[20] or into neural-like and glial-like cells[21]; they have been shown to have immunoregulatory properties[10,12-14,17,18,22,23]; they secrete factors that may stimulate endogenous neural stem cells in the CNS[24,25]; they can be safely injected autologously without the need for immunosuppression[26]; they decrease the number of infiltrating inflammatory cells, preserving axons and ameliorating demyelination,[15,19,27,28] ameliorate neuroinflammation[11,29] and exert a neurotrophic action.[16] All these features make MSC suitable for therapy in autoimmune diseases. In fact, the beneficial effects of MSC have been reported in EAE models.[25,30-34] Most of these studies were carried out in mice and in Lewis rats, and this means that to date the immunomodulatory effects of MSC in EAE Lewis 1A rats have not been studied. However, EAE Lewis 1A rats have been widely used to study the effects of new drug candidates for MS. Lewis 1A rats belong to the Major Histocompatibility Complex (MHC) congenic Lewis rat strain. The effects of MSC on T- and antigen-presenting cells have been studied in depth. Nevertheless, and contrary to their well-known effect on T-cells and antigen-presenting cells, the effect of MSC on B-cells remains unclear. B-cells are critical for myelin oligodendrocyte glycoprotein (MOG)-induced EAE, but are redundant in MOG (35-55)-induced EAE.[35,36] This is important, because B-cells play a critical role in human MS.[37] Moreover, MSC have been implicated in B-cell development in bone marrow, spleen and lymphoid follicles,[38] exerting a negative control on B-cell lymphopoiesis. In addition, it has been reported that B-cell proliferation is inhibited by MSC,[38] although this has not been confirmed by other authors.[39] The distribution of MSC in bone marrow and secondary lymphoid organs allows an intimate interaction between both cell subsets, which contributes to normal lymph node development[40] as well as to the support of tumor B-cells in follicular lymphomas.[41] Therefore, the study of the effect of MSC on B-lymphocytes is necessary to increase our insight into autoimmune processes such as MS. In addition to the demonstration of the immunomodulatory effects of MSC on B- and T-lymphocytes in the EAE model, the effects of such cells on the abolition or not of EAE episodes must be studied. Another important point to make clear is the expression of α4 integrins. In this sense, murine bone marrow (BM)-MSC do not express α4 integrins,[42] and one study on BM-MSC focusing on EAE lesions has provided conflicting results regarding the capacity of BM-MSC to migrate into inflammed CNS.[25,30-32,43] In a mouse model of EAE, it has been reported the synergic effect (e.g., the expression of the brain-derived neurotrophic factor was increased) of BM-MSC when these cells were combined with fasudil[44] (this combination reduced the severity of EAE in comparison with fasudil or BM-MSC alone); that rapamycin increased the immunomodulatory properties of BM-MSC,[45] playing the latter combination an important role in neuroprotection, and that the combination of resveratrol and BM-MSC increased the immunomodulatory effects (pro-inflammatory cytokines were suppressed and anti-inflammatory ones were increased).[46] Moreover, in a rat model of EAE, it has been demonstrated that the combination therapy of BM-MSC and EGb761 (a *Ginkgo biloba* extract) increased the neuroprotective effects, inhibited the secretion of pro-inflammatory cytokines and decreased the disease severity and the inflitrated cells.[47] Finally, in a mouse model of EAE, it has been reported that MSC controlled the induction of T-cells with a regulatory phenotype and the inhibition of pro-inflammatory T-cells.[48]

In the light of the foregoing data, we tested the therapeutic potential of intravenously injected BM-MSC from healthy donors in a chronic EAE model (Lewis 1A rats). We studied *in vitro* the immunomodulatory effects of MSC on the B- and T-lymphocytes of both control and EAE animals and the effects of intravenously administered MSC on EAE episodes.

## MATERIALS AND METHODS

### Animals and Induction of EAE

Lewis 1A female rats (n=23) aged 10-11 weeks (weight around 190 g) obtained from CERJ Janvier (France) were used. The animals were kept under standardized lighting and temperature conditions and had free access to food and water. They remained for at least ten days in their cages before the experiments. The aminals were weighed and scored according to the scale described below in "Animal groups and clinical evaluation of EAE" 6 days/week throughout the experiments. The experimental design, protocols, and procedures were performed under the guidelines of the ethical and legal recommendations of Spanish and European law. The study was also approved by the experimental research commission of the University of Salamanca (Spain).

EAE rats were immunized with a solution containing 50 µg of MOG and complete Freund's adjuvant (ACF), to which heat-inactivated *Mycobacterium tuberculosis* H37RA had been added. The rats were anaesthetized with isofluorane and the solution was injected intradermally at the base of the tail.

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

### Animal Groups and Clinical Evaluation of EAE

Animals were classified in different groups: 1) Control animals (group 1; n=3); 2) EAE animals (group 2; n=5); 3) EAE animals to which MSC were administered before the induction of EAE (group 3; n=5); 4) EAE animals to which MSC were administered after the induction of EAE (group 4; n=5); and 5) EAE animals to which MSC were administered before and after the induction of the EAE (group 5; n=5).

The animals were weighed and scored according to a previously described scale[49] 6 days/week throughout the experiments. The neurological signs of EAE were assessed and scored using this scale: 0, no signs; 1, tail weakness or tail paralysis; 2, hind leg paraparesis or hemiparesis; 3, hind leg paralysis or hemiparalysis; 4, complete paralysis (tetraplegy). Any animal reaching a moribund state was immediately perfused (see below) in order to avoid suffering. At the end of the experiment, all the animals were deeply anaesthetized with urethane (1 g/kg, intraperitoneal) and perfused *via* the ascending aorta with 50-100 ml of cold physiologic saline (0.9% NaCl) and then with 500 ml of cold 4% paraformaldehyde in 0.1 M phosphate-buffer (pH 7.2).

### Cell Isolation

MSC were isolated from the femora and tibias of wild-type female Lewis 1A rats (aged 10-11). MSC were harvested and placed in culture in DMEM containing 10% fetal bovine serum and 1% penicillin streptomycin. Cells were cultured at 37º C in a humidified atmosphere in the presence of 5% $CO_2$. Cultures were incubated at a final concentration of 3 x $10^6$ nucleated cells per ml at 37° C in a 5% $CO_2$ humidified incubator for 72 h. Non-adherent cells were aspirated on day 3 and the adherent population was cultured to achieve the maximum number of fibroblast colony-forming units prior to initial passage. Twice a week, adherent cells were fed by complete replacement of the medium. When the layer was confluent, cells were detached by treatment with trypsin and then subcultured at a concentration of 10,000 cells/$cm^2$ until confluence. This process was repeated at least 3 times in order to obtain a sufficient number of cells for analysis. All cultures were used between the third and fifth passages.

Adipogenic, osteogenic and condrogenic differentiation was induced as previously described[50] and recommended by the International Society of Cell Therapy (ISCT) consensus.[51] Phenotypic characterization was performed using the following monoclonal antibody combinations: Fluorescein (FITC)/Phycoerythrin (PE)/Peridin chlorophil protein-Cyanine-5 (PerCP-Cy5)/Allo-phycocyanin (APC): CD45/CD106/CD90.1/MHC II. Data acquisition was performed with a FACScalibur™ flow cytometer and data analysis with the Paint-A-Gate program.

B- and T-lymphocytes were obtained from splenocytes from healthy donor rats using cell sorting. Cells were stained with CD45RA and CD3, following the manufacturer's instructions, for B- or T-cells, respectively. For isolation, a FACSAria Cell Sorting flow cytometer was used. Positively selected cells contained >95% B- or T-cells, as assessed by flow cytometry.

### Administration of MSC

Animals were deeply anaesthetized with isofluorane. MSC were administered intravenously in the tail vein. Depending on the animal group (3, 4 or 5), the rats were infused at the base of the tail with a solution of sterile phosphate-buffered saline (PBS) containing MSC. MSC were infused two weeks before the induction of EAE (groups 3 and 5); eighteen days after induction (groups 4 and 5); and five weeks after the induction of EAE (groups 4 and 5).

### Cell Viability Assays

$10^5$ MSC were seeded in the plates and after 12 hours, $10^6$ positively selected B- or T-lymphocytes were seeded in the 96-well culture plate. Cells were cultured for 3 days in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. The effect of MSC on B-cell or T-cell growth was assessed by measuring the 3-(4.5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) dye absorbance of the cells. For this, $10^5$ B-cells or T-cells/100 mL were plated in triplicate onto 96-well tissue culture dishes in culture medium with or without $10^4$ MSC. MTT absorbance was assessed on the third day. Three wells were analyzed for each condition, and the results were presented as means±SD of triplicates.

For the detection of apoptosis, an AnnexinV-PE/7 amino-actinomycin (7-AAD) apoptosis detection kit was used. A minimum of $10^6$ lymphocytes were washed and resuspended in binding buffer (1:10 diluted in PBS), maintaining a cell concentration of $1\times10^6$/mL. Annexin V-PE and 7-AAD, 5 mL each, were added for 15 minutes. In order to identify B- and T-lymphocytes, anti-CD45RA-FITC and anti-CD3-APC were also added. For each condition, 50,000 events were collected and analyzed. The samples were acquired using Trucount™ Tubes, which contain a calibrated number of fluorescent microbeads. The absolute count of annexin V-PE plus 7-AAD-negative cells was calculated using the following equation: (number of events in region containing annexin V-PE plus 7-AAD negative cells/number of events in the absolute count bead region) x (number of beads per-test/test volume). The Win MDI software was used for analysis.

### Proliferation Assays

Studies were also performed on B-lymphocytes and T-lymphocytes cell cycles and DNA contents. For this, $5\times10^5$ lymphocytes were cultured for 2 days. The cells were stained with 500 ml of solution B containing 0.5 g/L of RNAse; this solution was added for 10 minutes in the dark. Finally, 500 ml of solution C, con-

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

taining 0.42 g/L of propidium iodide, was added to each tube and the cells were incubated in the dark for 15 minutes. After this period, measurements of DNA cell contents were performed on a FACScalibur™ flow cytometer. A minimum of 20,000 events were acquired. For the analysis of the distribution of the cells along the cell cycle phases, the model included in the ModFit LT™ software program was used after excluding cell debris and cell doublets in a FSC/FL2 area and a FL2 width/FL2 area dot plot, respectively.

#### Immunophenotipic Characterization

$5\times10^4$ MSC were seeded in the chamber of a 48-transwell plate and after 12 hours, $5\times10^5$ positively selected B-lymphocytes were seeded in the chamber. The cells were cultured for 3 days in 1 mL of RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin.

T-cell activation was analyzed using the following monoclonal antibody combinations: FITC/PE/PerC-Cy5/APC: -/IL-4/-CD3, -/IFN-γ/-CD3. Standard intracellular cytokine staining was performed using a leukocyte activation cocktail—a ready-to-use polyclonal cell activation mixture with phorbol ester PMA (Phorbol 12-Myristate 13-Acetate), a calcium ionophore (ionomycin), and the protein transport inhibitor GolgiPlug™ (brefeldin A)—following the manufacturer's instructions. After two washes with staining buffer, samples were first stained extracellularly with anti-CD3 before they were fixed and permeabilized for intracellular staining with phycoerythrin (PE)-conjugated anti-IL-14 or PE-conjugated anti-IFN-γ. Isotype-matched PE- and APC-conjugated monoclonal antibodies (mAbs) of irrelevant specificity were tested as negative controls. B-cell maturation was analyzed using the following monoclonal antibody combinations: FITC/PE/PerC-Cy5/APC: CD45RA/-CD86/-CD27. After staining for the antigens, cells were washed and resuspended in 0.5 ml of PBS until their acquisition in the flow cytometer. Data acquisition was performed on a FACSCalibur™ flow cytometer using the CellQuest™ software program, gated on the live population, and was analyzed using the Win MDI software. Analysis of both intracellular cytokines and the percentage of cells positive for surface antigens was performed on the gated population using the CellQuest™ software.

## RESULTS

#### General Considerations

After analysing their morphology and phenotype, rat MSC can be said to fulfill the requirements established by the International Society of Cellular Therapy (ISCT).[51] Moreover, rat MSC expressed the vascular cell adhesion molecule CD106 (VCAM) to a slight extent; this could explain the migratory effect of MSC. MSC promoted T- and B-lymphocyte survival in all cases studied and did not modify the T- or B-lymphocyte cell cycles in a quiescent state. The results also confirmed that MSC modulated EAE through the production of soluble cytokines. That is, the presence of MSC increased IFN-γ production and decreased IL-4 production in a different way. Thus, *in vitro* MSC decreased EAE by immunomodulating the T-helper 1 (Th1)/Th2 response. The presence of MSC modulated CD27/CD86 expression; they increased CD27/CD86 expression on the T-cells of healthy animals to a certain extent, and they significantly decreased CD27/CD86 expression in EAE animals.

Regarding the clinical evaluation of the EAE, it was observed that either MSC decreased the EAE clinical score or the animals did not develop EAE when those cells were infused prior to the induction of EAE. Finally, it is important to note that in order to confirm the results found in this study more animals must be used and a detailed statistical analysis must also be conducted. This procedure could confirm some of the data shown in the results section of this preliminary study.

#### Isolation, Differentiation and Characterization of MSC

The isolation of BM-derived MSC was accomplished by culturing BM cells obtained from femora and tibias. MSC formed a heterogeneous population of cells that proliferated *in vitro* as plastic-adherent cells, had a fibroblast-like morphology, and formed colonies *in vitro*. After 2-3 passages, cells with a fibroblastic appearance reached confluence (Figure 1A, 1B). An enrichment in MSC was documented 1 month later by positive staining of cultured cells with anti-CD90.1 (Thy 1) and a slight expression of CD106 (V-CAM). However, they were negatively stained for CD45 (hematopoietic lineage marker) and MHC class II molecules (Figure 1C, 1D).

#### Immunomodulatory Effects of MSC on T- and B-Cells

To investigate the immunomodulatory effects of purified MSCs, myelin-sensitized lymphocytes (obtained from EAE rats and healthy donor rats) were cultured with MSC at 1:10 concentration. For this purpose, purified T- or B-lymphocytes were cultured for 48 or 72 hours with or without MSC in 96- or 48-well plates. MSC increased the viability of T- and B-cells in all cases studied. As shown in Figures 2 and 3, respectively, the presence of MSC increased T-cell (Figure 2) and B cell viability (Figure 3). Similar results were obtained in the rest of the experimental groups analysed after 3 days of culture. These results are shown in Table 1. Analyses with MTT confirmed those findings (data not shown) (Figures 4 and 5).

In order to analyse the production of soluble cytokines, the production of T-cell cytokines with or without MSC was studied. In the cases studied, MSC modulated the production of soluble cytokines in a different way. In control animals, MSC maintained or decreased the production of soluble IFN-γ, whereas in the EAE animals they increased its production (Figure 6). The opposite occurred with the production of IL-4 (Figure 7). Healthy T-lymphocytes secreted more IL-4 with MSC in the culture than EAE T-lymphocytes (Figure 8).

MSC modulated CD27/CD86 expression in B-cells. MSC increased the percentage of CD27-positive cells in healthy

NEURO
Open Journal

Openventio
PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

**Figure 1.** MSC Morphology at the Time of the First Passage (A), and at the Time of the Second Passage (B). MSC from Controls Proliferated and Acquired a Spindle-Shaped Morphology (arrow). C: Immunophenotypic Assays of MSC from Healthy Donors. R1 Represents the Selection of the Percentages of Viable MSC. D: Differential Expression of the Markers Analyzed between Controls (blue line) and the MSC Phenotype (red line).

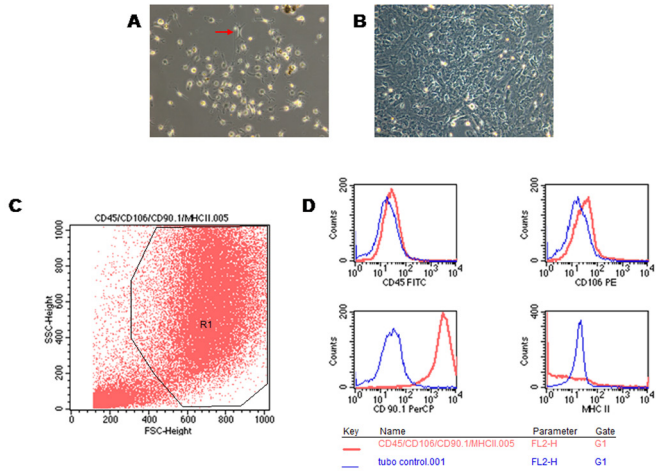



**Figure 2.** T-Cell Viability Assessed by Annexin/7AAD Staining in the Absence (A to C) or Presence (D to F) of MSC in some Cases Analysed. CD45RA cells were Excluded and CD3 Cells Positively Selected. The First Two Dot Blots are from the Healthy Donor Animal Group, the Second two from the EAE Animal Group, and the Third Two are from EAE Animals to which MSC were Administered before and after the Induction of EAE. Percentage of Annexin/7ADD-Positive Cells for Samples Cultured with (violet column) or without (maroon column) MSC. (1) Control animals (group 1); (2) EAE Animals (group 2); (3) EAE Animals to which MSC were Administered before the Induction of EAE (group 3); (4) EAE Animals to which MSC were Administered after the Induction of EAE (group 4); and (5) EAE Animals to which MSC were Administered before and after the Induction of EAE (Group 5) (G).

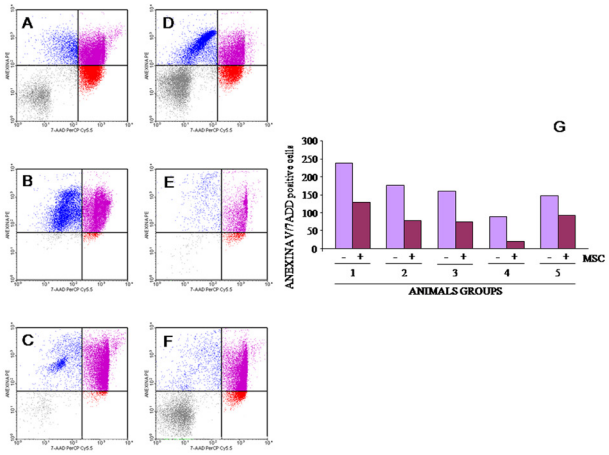



**Figure 3.** B-Cell Viability Assesed by Annexin/7AAD Staining after 4 Days of Culture Increased Significantly in the Presence of MSC. CD45RA Cells were Selected. B-Cell Viability Assessed by Annexin/7AAD Staining in the Absence (A to C) or Presence (D to F) of MSC in some Cases Analysed. The First Two Dot Blots are from the Healthy Donor Animal Group, the Second Two Dot Blots are from the EAE Animal Group, and the Third Two Dot Blots are from the EAE Animals to which MSC were Administered before and after the Induction of EAE. G: Annexin/7ADD-Positive Cells for Samples Cultured with Violet Column) or without (Maroon Column) MSC: (1) Group 1; (2) Group 2; (3) Group 3; (4) Group 4; and (5) Group 5 of Experimental Animals.

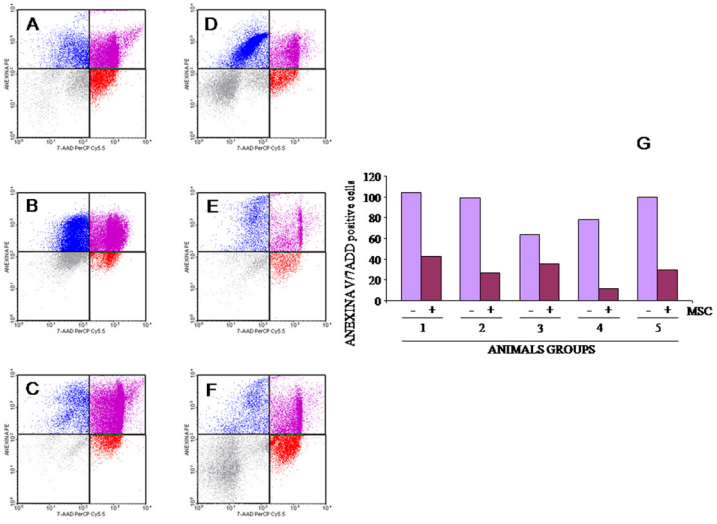



**Table 1:** Among these Cells the Number of Events Negative for both Annexin and 7-AAD was Calculated. A Calibrated Number of Microbeads was used.

| | 1- MSC | 1+ MSC | 2- MSC | 2+ MSC | 3- MSC | 3+ MSC | 4- MSC | 4+ MSC | 5- MSC | 5 + MSC |
|---|---|---|---|---|---|---|---|---|---|---|
| ANEXIN V+/7AAD+ | 103.88 | 42.56 | 98.23 | 26.49 | 63.46 | 35.53 | 77.62 | 11.04 | 99.02 | 29.40 |
| VIABLE | 23.29 | 49.13 | 10.48 | 11.12 | 8.45 | 6.95 | 15.72 | 3.46 | 3.69 | 25.90 |

T- or B-cell culture induced a low proliferation, fewer than 5% of cells being in the S or G2M phases of the cell cycle. Culture with MSC does not modify the cell cycle of T (Figure 4) or B (Figure 5) cells in a quiescent state. Similar results were obtained in the rest of the experimental groups analysed after 3 days of culture, as shown in Table II.

NEURO
Open Journal

Openventio
PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

**Figure 4:** Cell Cycle of T-Cells Cultured in the Absence of MSC (A-B) or in the Presence of MSC (C-D). The First Two Dot Blots are from Group 1 of the Experimental Animals, and the Next Dot Blots are from Group 5 of the Experimental Animals. No Changes in the T-Cell Cycle were Observed in any Case.

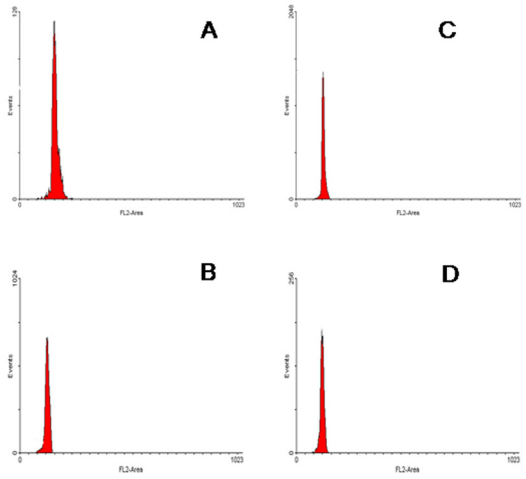



**Figure 5:** The same as in Figure 4 but Referring to the B-Cell Cycle.

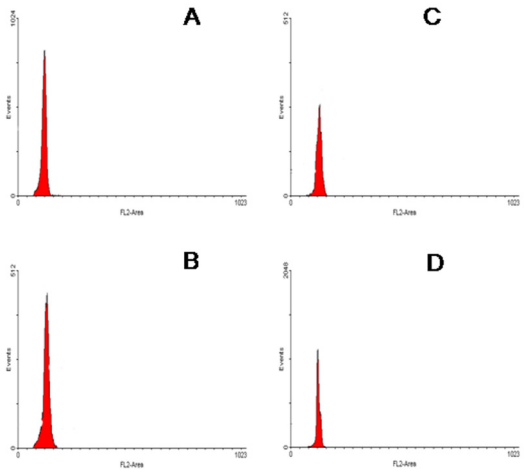



**Figure 6:** Percentage of IFN-g (Blue E)-Positive T-Cells after 3 Days of Culture without (A-C) MSC or with (D-F) MSC. Group 1 of the Experimental Animals (A, D), Group 3 of the Experimental Animals (B, E), and Group 5 of the Experimental Animals (C, F).

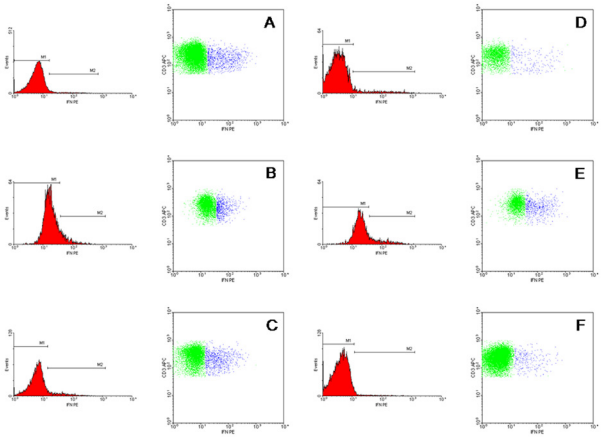



**Figure 7:** Percentage of IL-4 (Blue Events)-Positive T-Cells after 3 Days of Culture without (A-C) MSC or with (D-F) MSC. Purified CD3-Positive Cells were Analysed. The First Two Blots are from Group 1 of the Experimental Animals, the Second Two Blots are from Group 3 of the Experimental Animals, and the Third Two Blots are from Group 5 of the Experimental Animals.

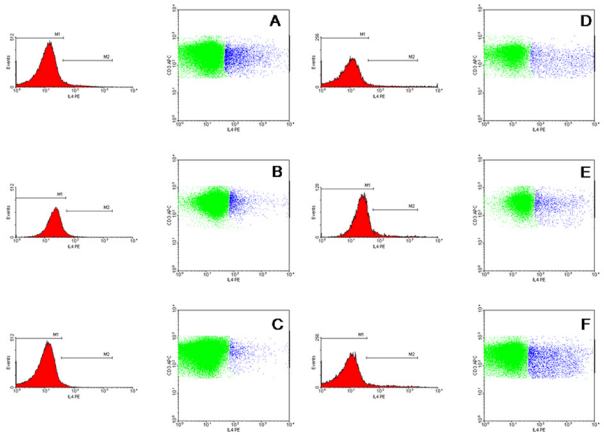
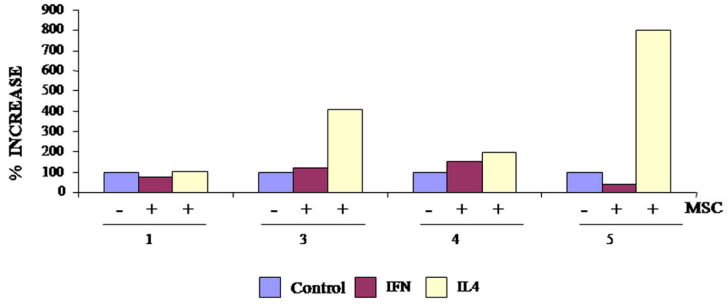



**Figure 8:** Increase in Positive Cells After the Culture of T-lymphocytes in the Absence of MSC (Violet Column) or in Presence of MSC for IFN-γ (Maroon Column) or IL-4 (Yellow Column), for the Different Cases Analysed. (1) Group 1; (3) Group 3; (4) Group 4; and (5) Group 5 of Experimental Animals.



Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

donors, whereas MSC significantly decreased CD27 expression in animals in which the clinical score was decreased due to MSC infusion (Figure 9). The same occurred with CD86 expression (Table 2).

### Clinical Evaluation of EAE

Non-EAE immunized animals (group 1) showed a clinical score of 0. However, EAE-induced animals (groups 2-5) developed a mean clinical score of 2.2, with a standard deviation of 0.7 (the animals that did not develop the disease were not considered in the statistical data: one animal from group 3 and another animal from group 5). It is noteworthy that the animals infused with MSC prior to EAE immunization either failed to develop EAE or their EAE clinical scores were reduced. In contrast, the animals that received MSC after the induction of EAE developed a normal course of EAE.

In sum, rat MSC expressed the vascular cell adhesion molecule CD106 to a slight extent. MSC promoted T- or B-lymphocyte survival but did not modify the T- or B-lymphocyte cell cycles in the quiescent state. The results also confirmed that MSC modulated EAE though the production of soluble cytokines. *In vitro,* MSC decreased the EAE by immunomodulating the Th1/Th2 response. Moreover, MSC controlled CD27/CD86 expression in different ways. Animals infused with MSC prior to the EAE immunization did not develop EAE, or their EAE clinical scores were decreased, whereas animals that received MSC after

**Figure 9:** Analysis of CD27/CD86-Positive B-Cells in the Absence of MSC (A-C) or in the Presence of MSC (D-F) for 3 Days of Culture. The First Two Dot Blots are from Healthy Donor; the Next Ones are from EAE Experimental Animals and the Last Ones are from EAE Animal to which MSC were Administered before and after the Induction of EAE. G: Increase in Positive Cells after Culture of B Lymphocytes in the Absence of MSC (Violet Column) or in the Presence of MSC (Maroon Column) for CD27/CD86 Expression: (1) Group 1; (2) Group 2; (3) Group 3; (4) Group 4; and (5) Group 5 of the Experimental Animals.

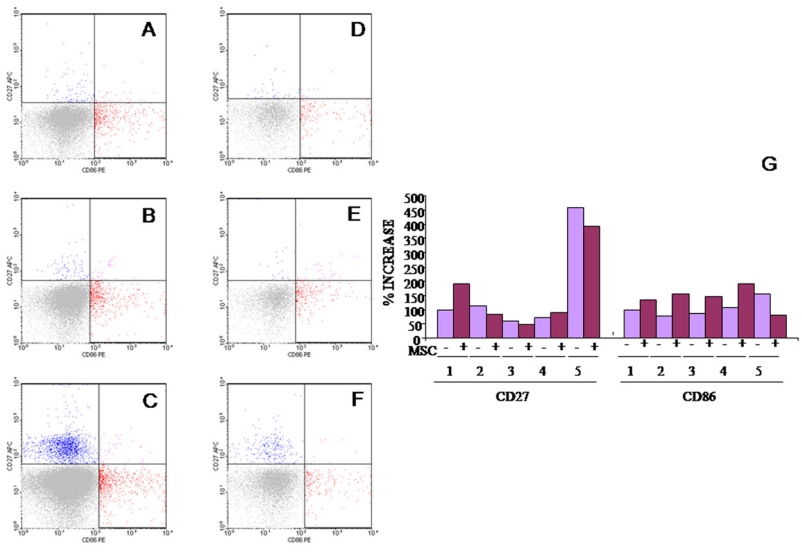



**Table 2:** The Results Show, Respectively, the Different Phases of the Cell Cycle for T- or B-cells. In all the Cases not even 5% of the Cells were in S Phase and hence the Presence of MSC did not Modify the Cell Cycle in a Quiescent State.

| T-Lymphocytes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1- MSC | 1+ MSC | 2- MSC | 2+ MSC | 3- MSC | 3+ MSC | 5- MSC | 5+ MSC |
| G0/G1 | 88.25 | 96.9 | 96.02 | 98.9 | 92.4 | 97.22 | 97.25 | 96.64 |
| S | 1.71 | 1.78 | 3.29 | 2.05 | 0.15 | 0.41 | 0.01 | 0.03 |
| G2/M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **B-Lymphocytes** | | | | | | | | |
| | 1- MSC | 1+ MSC | 2- MSC | 2+ MSC | 3- MSC | 3+ MSC | 5- MSC | 5+ MSC |
| G0/G1 | 92.7 | 93.37 | 96.94 | 98.95 | 97.62 | 94.9 | 90.55 | 96.66 |
| S | 0.73 | 0.05 | 2.09 | 0 | 0.36 | 0.78 | 4.24 | 0.78 |
| G2/M | 0.26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

the induction of EAE developed a normal EAE course.

## DISCUSSION

MSC belong to a more recent field in the range of experimental therapies currently being developed to treat MS. While interest in the use of MSC was originally due to their potential capacity to differentiate into different cell lineages, recent work demonstrating their interesting immunological properties has led to a revised concept, envisaging their use for immunomodulatory purposes. These properties have already been exploited in the clinical setting for the treatment of severe autoimmune diseases. It has been reported that MSC inhibit monocyte-derived dendritic cell maturation as well as T-lymphocyte proliferation.[52-55] MSC have been reported to inhibit the proliferation of T-cells through an MHC-independent mechanism,[56] leading to the arrest of cell division.[53] Although, the mechanisms involved in this inhibition of T-cell proliferation are still poorly understood, a veto-like activity has been reported,[57] and a role for soluble molecules—including prostaglandin E2,[54] transforming growth factor,[58] and indoleamine 2,3-dioxygenase[59]—has been proposed. It has been reported that MSC increase the survival of unstimulated T-cells and inhibit the proliferation of activated T-cells.[60] This is in accordance with previous studies indicating that MSC arrest T-cells in the G1 phase of the cell cycle[53] and that this effect is mediated by the inhibition of cyclin D2 and the upregulation of p27kip1.

It is known that MSC inhibit B-cell proliferation and differentiation[61]; that MSC may induce both the expansion and differentiation of B-cells stimulated with an agonist of the Toll-like receptor (TLR) 9 in the absence of B cell receptor triggering[62]; and that MSC increase antibody secretion by human B-cells stimulated with lipopolysaccharide, cytomegalovirus, or varicellazoster virus.[63] More recently, it has been described that MSC promote the survival and inhibit the proliferation and maturation of B-cells.[64] These effects are mediated through the activation of MAPK pathways such as pErk 1/2 and p38. Here, it has been shown that MSC play a critical role in the immune regulation in EAE. Thus, we provide evidence that MSC given to Lewis 1A rats can, *in vitro,* support cell survival when T- or B-cells are in a quiescent state, leading to cell apoptosis. First, we observed that T- or B-cells cultured under non-stimulated conditions, a cell population physiologically prone to spontaneous cell death, are significantly rescued from apoptosis by the presence of MSC. Other authors have suggested that the protective effect of MSC mainly targets the "death receptor" pathway of apoptosis, through the downregulation of the Fas receptor and the Fas ligand on arrested T-cells.[60] In this sense, and in the same manner, the increased viability induced by MSC in resting B-cells could explain the higher production of IgG upon weak stimulation, as shown in previous studies.[63,64] However, we analysed the effect of MSC on T- or B-cell cycles and, contrary to the results showing that MSC can hamper T-cell proliferation through the inhibition of cell division and subsequent accumulation of cells in the G0 phase of the cell cycle,[65] we did not find any difference between T- or B-cells cultured with or without MSC. This is also in part due to the absence of stimulating conditions and the low T- or B-cell proliferation capacity in the quiescent state.

However, there is a marked increase in the expression of TLR in MS brain lesions and cerebrospinal fluid mononuclear cells as well as in EAE brain lesions.[66,67] TLR3 signals cause Th1 polarization with increased IFN-γ secretion concomitant with increased CD4 T-cell death.[68] TLR signals are therefore potent modulators of microglial activation programs. The MSC-induced improvement was accompanied by changes in neural cell responses, with increased oligodendrocytes and decreased astrocytes in lesioned areas as well as changes in spleen cell responses.[32] In active EAE, the predominant response is mediated through Th1 pro-inflammatory cells and the expression of their associated cytokines. In animals that received human BM-MSC, there was a significant reduction in pro-inflammatory cytokines, including IL-17, IFN-γ, IL-2, IL-12p70, and TNF-α, and a significant increase in anti-inflammatory cytokines, including IL-4 and IL-5. Moreover, it has been reported that MSC can suppress the T-cell proliferative response against TCR-dependent and -independent polyclonal stimuli.[43] Such an effect was paralleled by a significant suppression of IFN-γ and TNF-α production by activated T-cells, supporting the notion of a profound inhibition of the Th1 response by MSC. Compatible with this, we observed that MSC regulated the balance of T lymphocytes between Th1/Th2 and modified the cytokines released during EAE. Thus, the presence of MSC increased the production of IFN-γ by T-cells in the EAE animals. However, this increase was not seen in the healthy animals or in the EAE animals belonging to experimental group 5 (animals to which MSC were administered before and after the induction of the EAE). Moreover, the production of IL-4 increased in all cases analysed. The most important aspect was the increase in the production of soluble IL-4 by T-cells in the EAE animals of group 5. This means that MS is a T-cell-mediated autoimmune disease, involving inflammatory demyelination of the CNS by CD4+ T-cells specific for myelin oligodendrocyte glycoprotein and other CNS autoantigens.[69]

In recent years, B-cells have emerged as a novel therapeutic target for treating MS, and clinical data with rituximab, as a B cell-depleting monoclonal antibody-based therapy, provide reciprocal conceptual support for a prominent role of B-cells in the pathogenesis of MS.[70] Memory B-cells are significantly different from naïve B-cells, and the production of different effector cytokine profiles is a fundamental characteristic distinguishing naïve (CD27−) from memory (CD27+) human B-cells.[71] Memory and naïve B-cells are considered to play different roles in immune regulation. However, the roles of memory and naïve B-cell subsets in MS have not yet been elucidated. Moreover, CD80 and CD86 are major costimulatory signals for T-cell activation, and variations in the expression of these proteins are likely to influence immune regulation in MS.[72] Here, we examined the expression of CD86 and CD27 in B-cells by flow cytometry. In this regard, a high expression of CD27/CD86

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

was observed in the EAE animals of experimental group 5 *versus* healthy animals. In culture, this increment decreased in the presence of MSC. In the present study, we observed that subsets of memory and naïve B-cells differ between EAE animals and healthy control animals, and these differences could be exploited in the search for targets in MS therapies. Taken together, these results suggest that naïve and memory B-cell subsets play different active roles in the regulation of normal immune responses and indicate that abnormalities in these functions may contribute to MS. Moreover, the effect of MSC on B-cells antigen expression suggested that MSC could modulate the disease, which partly depended on the day on which the MSC were injected.

Finally, the clinical course of EAE was significantly ameliorated in the animals treated with MSC (intravenous administration). In this sense, it seems that the beneficial effects of MSC only appear if these cells are infused prior to the induction of EAE.

## ACKNOWLEDGEMENTS

This work has been supported by Gemacbio (Saint Jean d'Illac, France), the Institut pour le Developpement de la Recherche en Pathologie Humaine et Therapeutique (IDRPHT, Talence, France), Red de Terapia Celular de Castilla y León (Spain), Consejería de Educación (Junta de Castilla y León, Spain) (SA099A08) and the INCYL-Federación de Cajas de Ahorro de Castilla y León (Spain). Arturo Mangas was supported by the Red de Terapia Celular de Castilla y León (Spain). The authors wish to thank to Professor Javier Yajeya (University of Salamanca, Spain) for his help in the studies carried out on the sciatic nerve motor conduction velocity. The authors also wish to thank Nicholas Skinner (University of Salamanca, Spain) for supervising the English text.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Cao Q, Benton RL, Whittemore SR. Stem cell repair of central nervous system injury. *J Neurosci Res*. 2002; 68(5): 501-510. doi: 10.1002/jnr.10240

2. McDonald JW, Howard MJ. Repairing the damaged spinal cord: A summary of our early success with embryonic stem cell transplantation and remyelination. *Prog Brain Res*. 2002; 137: 299-309. doi: 10.1016/S0079-6123(02)37023-7

3. Campos LS. Neurospheres: Insights into neural stem cell biology. *J Neurosci Res*. 2004; 78: 761-769. doi: 10.1002/jnr.20333

4. Reubinoff BE, Itsykson P, Turetsky T, et al. Neural progenitors from human embryonic stem cells. *Nat Biotech*. 2001; 19: 1134-1140. doi: 10.1002/jnr.20333

5. Oyama Y, Craig RM, Traynor AE, et al. Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. *Gastroenterology*. 2005; 128(3): 552-563. doi: 10.1053/j.gastro.2004.11.051

6. Mahmood A, Lu D, Wang L, Chopp M. Intracerebral transplantation of marrow stromal cells cultured with neurotrophic factors promotes functional recovery in adult rats subjected to traumatic brain injury. *J Neurotrauma*. 2002; 19(12): 1609-1617. doi: 10.1089/089771502762300265

7. Akiyama Y, Radtke C, Kocsis JD. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. *J Neurosci*. 2002; 22: 6623-6630.

8. Pluchino S, Quattrini A, Brambilla E, et al. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature*. 2003; 422: 688-694. doi: 10.1038/nature01552

9. Pluchino S, Zanotti L, Rossi B, et al. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature*. 2005; 436: 266-271. doi: 10.1038/nature03889

10. Anderson P, González-Rey E, O'Valle F, Martín F, Oliver FJ, Delagado M. Allogeneic adipose-derived mesenchymal cells ameliorate experimental autoimmune encephalomyelitis by regulating self-reactive T cell reponses and dendritic cell function. *Stem Cells Int*. 2017. doi: 10.1155/2017/2389753

11. Marín-Bañasco C, Benabdellah K, Melero-Jerez C, et al. Gene therapy with mesenchymal stem cells expressing IFN-β ameliorates neuroinflammation in experimental models of multiple sclerosis. *Br J Pharmacol*. 2017; 174: 238-253. doi: 10.1111/bph.13674

12. Mohammadzadeh A, Pourfathollah AA, Shahrokhi S, et al. Evaluation of AD-MSC (adipose-derived mesenchymal stem cells) as a vehicle for IFN-β delivery in experimental autoimmune encephalomyelitis. *Clin Immunol*. 2016; 169: 98-106. doi: 10.1016/j.clim.2016.06.015

13. Yousefi F, Ebtekar M, Soudi S, Soleimani M, Hashemi SM. In vivo immunomodulatory effects of adipose-derived mesenchymal stem cells conditioned medium in experimental autoimmune encephalomyelitis. *Immunol Lett*. 2016; 172: 94-105. doi: 10.1016/j.imlet.2016.02.016

14. Shalaby SM, Sabbah NA, Saber T, Abdel Hamid RA. Adipose-derived mesenchymal stem cells modulate the immune response in chronic experimental autoimmune encephalomyelitis model. *IUBMB Life*. 2016; 68: 106-115. doi: 10.1002/iub.1469

15. Jiang H, Zhang Y, Tian K, Wang B, Han S. Amelioration of experimental autoimmune encephalomyelitis through transplantation of placenta-derived mesenchymal stem cells. *Sci Rep*. 2017. doi: 10.1038/srep41837

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

16. Selim AO, Selim SA, Shalaby SM, Mosaad H, Saber T. Neuroprotective effects of placenta-derived mesenchymal stem cells in a rat model of experimental autoimmune encephalomyelitis. *Cytotherapy*. 2016; 18: 1100-1013.

17. Torkaman M, Ghollasi M, Mohammadnia-Afrouzi M, Salimi A, Amari A. The effect of transplanted human Wharton's jelly mesenchymal stem cells treated with IFN-γ on experimental autoimmune encephalomyelitis. *Cell Immunol*. 2017; 311: 1-12. doi: 10.1016/j.cellimm.2016.09.012

18. Bravo B, Gallego MI, Flores AI, et al. Restrained Th17 response and myeloid cell infiltration into the central nervous sytem by human decidua-derived mesenchymal stem cells during experimental autoimmune encaphalomyelitis. *Stem Cell Res Ther*. 2016; 7: 43. doi: 10.1186/s13287-016-0304-5

19. Rafieemehr H, Kheyrandish M, Soleimani M. Neuroprotective effects of transplanted stromal cells-derived human umbilical cord blood neural progenitor cells in EAE. *Iran J Allergy Asthma Immunol*. 2015; 14(6): 596-604.

20. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*. 1997; 276: 71-74.

21. Bossolasco P, Cova L, Calzarossa C, et al. Neuro-glial differentiation of human bone marrow stem cells in vitro. *Exp Neurol*. 2005; 193: 312-325. doi: 10.1016/j.expneurol.2004.12.013

22. Krampera M, Pasini A, Pizzolo G, Cosmi L, Romagnani S. Annunziato F. Regenerative and immunomodulatory potential of mesenchymal stem cells. *Curr Opin Pharmacol*. 2006; 6: 435-441. doi: 10.1016/j.coph.2006.02.008

23. Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: Central role of MSC-derived prostaglandin E2. *Blood*. 2009; 113: 6576-6583. doi: 10.1182/blood-2009-02-203943

24. Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. *Lancet Neurol*. 2002; 1: 92-100.

25. Zhang J, Li Y, Chen J, et al. Human bone marrow stromal cell treatment improves neurological functional recovery in EAE mice. *Exp Neurol*. 2005; 195: 16-26. doi: 10.1016/S1474-4422(02)00040-6

26. Bartholomew A, Polchert D, Szilagyi E, Douglas GW, Kenyon N. Mesenchymal stem cells in the induction of transplantation tolerance. *Transplantation*. 2009; 87: S55-S57. doi: 10.1097/TP.0b013e3181a287e6

27. Liao W, Pham V, Liu L, et al. Mesenchymal stem cells engineered to express selectin ligands and IL-10 exert enhanced therapeutic efficacy in murine experimental autoimmuneencephalomyelitis. *Biomaterials*. 2016; 77: 87-97. doi: 10.1016/j.biomaterials.2015.11.005

28. Gordon D, Pavlovska G, Uney JB, Wraith DC, Scolding NJ. Human mesenchymal stem cells infiltrate the spinal cord, reducede myelination, and localize to white matter lesions in experimental autoimmune encephalomyelitis. *J Neuropathol Exp Neurol*. 2010; 69: 1087-1095. doi: 10.1097/NEN.0b013e3181f97392

29. Hou Y, Heon Ryu C, Jun JA, Kim SM, Jeong CH, Jeun SS. Interferon β-secreting mesenchymal stem cells combined with minocycline attenuate experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 2014; 274: 20-27. doi: 10.1016/j.jneuroim.2014.06.001

30. Gerdoni E, Gallo B, Casazza S, et al. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. *Ann Neurol*. 2007; 61: 219-227. doi: 10.1016/j.jneuroim.2014.06.001

31. Kassis I, Grigoriadis N, Gowda-Kurkalli B, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. *Arch Neurol*. 2008; 65: 753-761. doi: 10.1001/archneur.65.6.753

32. Bai L, Lennon DP, Eaton V, et al. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia*. 2009; 57: 1192-1203. doi: 10.1002/glia.20841

33. Rafei M, Campeau PM, Aguilar-Mahecha A, et al. Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner. *J Immunol*. 2009; 182: 5994-6002. doi: 10.4049/jimmunol.0803962

34. Liu XJ, Zhang JF, Sun B, et al. Reciprocal effect of mesenchymal stem cell on experimental autoimmune encephalomyelitis is mediated by transforming growth factor-beta and interleukin-6. *Clini Exp Immunol*. 2009; 158: 37-44. doi: 10.1111/j.1365-2249.2009.03995.x

35. Iglesias A, Bauer J, Litzenburger T, Schubart A, Linington C. T- and B-cell responses to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis and multiple sclerosis. *Glia*. 2001; 36: 220-234. doi: 10.1002/glia.1111

36. Lyons JA, San M, Happ MP, Cross AH. B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide. *Eur J Immunol*. 1999; 29: 3432-3439. doi: 10.1002/(SICI)1521-4141(199911)29:11<3432::AID-IMMU3432>3.0.CO;2-2

37. McLaughlin KA, Wucherpfennig KW. B cells and autoantibodies in the pathogenesis of multiple sclerosis and related inflammatory demyelinating diseases. *Adv Immunol*. 2008; 98: 121-149. doi: 10.1016/S0065-2776(08)00404-5

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

38. Shoham T, Parameswaran R, Shav-Tal Y, Barda-Saad M, Zipori D. The mesenchymal stroma negatively regulates B cell lymphopoiesis through the expression of activin A. *Ann NY Acad Sci*. 2003; 996: 245-260. doi: 10.1111/j.1749-6632.2003.tb03253.x

39. Krampera M, Cosmi L, Angeli R, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells*. 2006; 24: 386-398. doi: 10.1634/stemcells.2005-0008

40. Nishikawa S, Honda K, Vieira P, Yoshida H. Organogenesis of peripheral lymphoid organs. *Immunol Rev*. 2003; 195: 72-80. doi: 10.1034/j.1600-065X.2003.00063.x

41. Amé-Thomas P, Maby-El HH, Monvoisin C, et al. Human mesenchymal stem cells isolated from bone marrow and lymphoid organs support tumor B-cell growth: Role of stromal cells in follicular lymphoma pathogenesis. *Blood*. 2007; 109: 693-702. doi: 10.1182/blood-2006-05-020800

42. De Ugarte DA, Alfonso Z, Zuk PA, et al. Differential expression of stem cell mobilization-associated molecules on multilineage cells from adipose tissue and bone marrow. *Immunol Lett*. 2003; 89: 267-270. doi: 10.1016/S0165-2478(03)00108-1

43. Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood*. 2005; 106: 1755-1761. doi: 10.1182/blood-2005-04-1496

44. Yu JW, Li YN, Song GB, et al. Synergistic and superimposed effect of bone marrow-derived mesenchymal stem cells combined with fasudil in experimental autoimmune encephalomyelitis. *J Mol Neurosci*. 2016; 60: 486-497. doi: 10.1007/s12031-016-0819-3

45. Togha M, Jahanshahi M, Alizadeh L, et al. Rapamycin augments immunomodulatory properties of bone marrow-derived mesenchymal stem cells in experimental autoimmune encephalomyelitis. *Mol Neurobiol*. 2017; 54: 2445-2457. doi: 10.1007/s12035-016-9840-3

46. Wang D, Li SP, Fu JS, Bai L, Guo L. Resveratrol augments therapeutic efficiency of mouse bone marrow mesenchymal stem cell-based therapy in experimental autoimmune encephalomyelitis. *Int J Dev Neurosci*. 2016. doi: 10.1016/j.ijdevneu.2016.01.005

47. Hao F, Li A, Yu H, et al. Enhanced neuroprotective effects of combination therapy with bone marrow-derived mesenchymal stem cells and Ginkgo biloba extract (EGb761) in a rat model of experimental autoimmune encephalomyelitis. *Neuroimmunomodulation*. 2016; 23: 41-57. doi: 10.1159/000437429

48. Luz-Crawford P, Kurte M, Bravo-Alegría J, et al. Mesenchymal stem cells generate a CD4+CD25+Foxp3+ regulatory T cell population during the differentiation process of Th1 and Th17 cells. *Stem Cell Res Ther*. 2013; 4: 65. doi: 10.1186/scrt216

49. Weissert R, Wallstrom E, Storch MK, et al. MHC haplotype-dependent regulation of MOG-induced EAE in rats. *J Clin Invest*. 1998; 102: 1265-1273. doi: 10.1186/scrt216

50. Villarón EM, Almeida J, López-Holgado N, et al. Mesenchymal stem cells are present in peripheral blood and can engraft after allogeneic hematopoietic stem cell transplantation. *Haematologica*. 2004; 89(12): 1421-1427.

51. Dominici M, Le BK, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; 8: 315-317.

52. Rasmusson I, Ringden O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. *Exp Cell Res*. 2005; 305: 33-41. doi: 10.1016/j.yexcr.2004.12.013

53. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood.* 105: 2821-2827. doi: 10.1182/blood-2004-09-3696

54. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005; 105: 1815-1822. doi: 10.1182/blood-2004-04-1559

55. Jiang XX, Zhang Y, Liu B, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood*. 2005; 105: 4120-4126. doi: 10.1182/blood-2004-02-0586

56. Le BK, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scan J Immunol*. 2003; 57: 11-20. doi: 10.1046/j.1365-3083.2003.01176.x

57. Potian JA, Aviv H, Ponzio NM, Harrison JS, Rameshwar P. Veto-like activity of mesenchymal stem cells: Functional discrimination between cellular responses to alloantigens and recall antigens. *J Immunol*. 2003; 171: 3426-3434. doi: 10.4049/jimmunol.171.7.3426

58. Di Nicola M, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood*. 2002; 99: 3838-3843. doi: 10.1182/blood.V99.10.3838

59. Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell

Openventio PUBLISHERS

ISSN 2377-1607 http://dx.doi.org/10.17140/NOJ-4-128

responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood*. 2004; 103: 4619-4621. doi: 10.1182/blood-2003-11-3909

60. Benvenuto F, Ferrari S, Gerdoni E, et al. Human mesenchymal stem cells promote survival of T cells in a quiescent state. *Stem Cells*. 2007; 25: 1753-1760. doi: 10.1634/stemcells.2007-0068

61. Corcione A, Benvenuto F, Ferretti E, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood*. 2006; 107: 367-372. doi: 10.1182/blood-2005-07-2657

62. Traggiai E, Volpi S, Schena F, et al. Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. *Stem Cells*. 2008; 26: 562-569. doi: 10.1634/stemcells.2007-0528

63. Rasmusson I, Le BK, Sundberg B, Ringden O. Mesenchymal stem cells stimulate antibody secretion in human B cells. *Scan J Immunol*. 2007; 65: 336-343. doi: 10.1111/j.1365-3083.2007.01905.x

64. Tabera S, Pérez-Simón JA, Díez-Campelo M, et al. The effect of mesenchymal stem cells on the viability, proliferation and differentiation of B-lymphocytes. *Haematologica*. 2008; 93: 1301-1309. doi: 10.3324/haematol.12857

65. Krampera M, Glennie S, Dyson J, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood*. 2003; 101: 3722-3729. doi: 10.1182/blood-2002-07-2104

66. Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of Toll-like receptors in the human central nervous system. *J Neuropathol Exp Neurol*. 2002; 61: 1013-1021. doi: 10.1093/jnen/61.11.1013

67. Andersson A, Covacu R, Sunnemark D, Danilov AI, Dal BA, Khademi M. Pivotal advance: HMGB1 expression in active lesions of human and experimental multiple sclerosis. *J Leukoc Biol*. 2008; 84(5): 1248-1255. doi: 10.1189/jlb.1207844

68. Jack CS, Arbour N, Blain M, Meier UC, Prat A, Antel JP. Th1 polarization of CD4+ T cells by Toll-like receptor 3-activated human microglia. *J Neuropathol Exp Neurol*. 2007; 66: 848-859. doi: 10.1097/nen.0b013e3181492a7

69. Williams KC, Ulvestad E, Hickey WF. Immunology of multiple sclerosis. *J Clin Neurosci*. 1994; 2: 229-245.

70. Hauser SL, Waubant E, Arnold DL, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *New Eng J Med*. 2008; 358: 676-688. doi: 10.1056/NEJMoa0706383

71. Duddy M, Niino M, Adatia F, et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J Immunol*. 2007; 178: 6092-6099. doi: 10.4049/jimmunol.178.10.6092

72. Kuchroo VK, Das MP, Brown JA, et al. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: Application to autoimmune disease therapy. *Cell*. 1995; 80(5): 707-718. doi: 10.1016/0092-8674(95)90349-6