UNIVERSIDADE FEDERAL DE SANTA CATARINA
DEPARTAMENTO DE CLÍNICA MÉDICA
CURSO DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS

ASSOCIAÇÃO DAS CONCENTRAÇÕES SÉRICAS DE CITOCINAS E A MORTALIDADE HOSPITALAR DE PACIENTES COM TRAUMATISMO CRANIANO GRAVE

FLÁVIA MAHATMA SCHNEIDER SOARES

Florianópolis, fevereiro de 2011

UNIVERSIDADE FEDERAL DE SANTA CATARINA
DEPARTAMENTO DE CLÍNICA MÉDICA
CURSO DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS

FLÁVIA MAHATMA SCHNEIDER SOARES

ASSOCIAÇÃO DAS CONCENTRAÇÕES SÉRICAS DE CITOCINAS E A MORTALIDADE HOSPITALAR DE PACIENTES COM TRAUMATISMO CRANIANO GRAVE

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Médicas da Universidade Federal de Santa Catarina, como requisito parcial à obtenção do grau de de Mestre.
Orientador: Roger Walz

Florianópolis, fevereiro de 2011

Associação das concentrações séricas de citocinas e a mortalidade hospitalar de pacientes com traumatismo craniano grave

Flávia Mahatma Schneider Soares

Dissertação de mestrado apresentada à Coordenação do Programa de Pós-graduação em Ciências Médicas-PPGCM, do Centro de Ciências da Saúde-CCS, da Universidade Federal de Santa Catarina-UFSC, comorequisito parcial para obtenção do grau de Mestre em Ciências Médicas.

Banca Examinadora:

____

Prof. Felipe Dal Pizzol
(UNESC) - Pesq. 1C do CNPq
(Membro)

____

Profa. Alexandra Latini
(UFSC) - Pesq. 2 do CNPq
(Membro)

____

Prof. Roger Walz
(UFSC) - Pesq. 1B do CNPq
(Presidente)

____

Flávia Mahatma Schneider Soares
Mestranda

Orientador: Roger Walz

Florianópolis, 22 de fevereiro de 2011

"Existe somente uma idade para a gente ser feliz, somente uma época na vida de cada pessoa em que é possível sonhar e fazer planos e ter energia bastante para realizá-los a despeito de todas as dificuldades e obstáculos...
... Tempo de entusiasmo e coragem em que todo desafio é mais um convite à luta que a gente enfrenta com toda disposição de tentar algo NOVO, de NOVO e de NOVO, e quantas vezes for preciso. Essa idade tão fugaz na vida da gente chama-se PRESENTE e tem a duração do instante que passa."

Mário Quintana

AGRADECIMENTOS

Ao meu orientador, Roger, pela oportunidade, profissionalismo, sabedoria, amizade, compreensão, dedicação e pela fundamental contribuição no desenvolvimento deste trabalho. Tenhas certeza que aprendi muito contigo nesses anos de convívio.

Ao prezado professor André Bafica, pelos ensinamentos, pela segurança, rigor e excelente conduta profissional.

A Universidade Federal de Santa Catarina (UFSC), pela possibilidade de realizar esse trabalho de pesquisa.

Aos professores do Departamento de Ciências Médicas da UFSC, em especial ao prof Edelton Morato pelas oportunidades de aprendizagem no decorrer da Pós Graduação.

A CAPES e FAPESC, pelo auxílio financeiro.

Aos amigos e colegas do LIDI, especialmente a Nicole Menezes de Souza, obrigada pela amizade, companheirismo, ótima convivência, ensinamentos, eficiência, comprometimento e apoio constante. Vocês ficarão para sempre em meu coração.

Aos amigos do NUPNEC pelo aprendizado e companheirismo.

A minha irmã, Carol, pelo amor, apoio, incentivo, conselhos, amizade, lealdade e por estar sempre ao meu lado.

Ao meu pai, Dirceu, por ser meu exemplo de vida, por me dar apoio, direção, segurança e aprendizado. Obrigada, fundamentalmente, pelas lições de disciplina, seriedade, sabedoria e caráter, pelo incentivo incondicional, carinho, compreensão e amor.

A minha mãe e melhor amiga, Elisabeth, que acompanhou com carinho e estímulo, procurando amenizar a ansiedade em cada telefonema ou rosto desanimado, mantendo-me firme diante dos obstáculos. Obrigada pela compreensão, palavras, sorrisos e por todo teu amor.

RESUMO

O trauma cranio-encefálico (TCE) é uma causa mundial de morbidade e mortalidade. A concentração sanguinea de citocinas têm sido associada com TCE. Neste estudo, nós investigamos os níveis séricos de IL-2, IL-4, IL-5, IL-10, TNF-α e INF-γ como biomarcadores de gravidade da lesão cerebral traumática e sua associação com a mortalidade hospitalar.

As concentrações séricas de citocinas foram determinadas em média (IQ 25/75) de tempo de 10 (18/05) horas após o TCE, em 93 pacientes consecutivos admitidos no Hospital Governador Celso Ramos. Para as comparações, foram selecionados aleatoriamente pacientes com TCE leve(n = 18) e moderado (n = 16), atendidos em nossa emergência. Em pacientes com TCE grave, 2 amostras adicionais de sangue foram analisadas 30 (22/37) e 68 (55/78) horas após a lesão. Os dados coletados incluíram idade, sexo, achados tomográficos, CSG de admissão e as reações da pupila, presença de trauma e a mortalidade hospitalar associada.

Mesmo em casos graves, os soros dos pacientes permaneceram indetectáveis para as citocinas IL-2, IL-4, IL-5 e IFN-γ após o TCE, por citometria de fluxo. Os níveis de IL-10, mas não os de TNF-α, correlacionam-se significativamente com a gravidade da GCS (p = coeficiente de 0,42, Sperman <0,0001) e foram associadas com a mortalidade hospitalar de pacientes com TCE grave. A elevação dos níveis séricos de IL-10 permaneceram significativamente associados com a mortalidade (p = 0,01) no subgrupo de pacientes com TCE grave isolado (n = 74). A análise de regressão logística mostrou que a concentração dos níveis séricos de IL-10 (> 0,9 pg/ml), medidos 10 ou 30 horas após a lesão, foram, respectivamente, 6 vezes (OR 6,2, IC 95% 1,2-25,1, p = 0,03) e 5 vezes (OR 5,4, IC 95% 1,2-25,1, p = 0,03) mais associados com a mortalidade do que os níveis mais baixos (<0,05 pg/ml), independentemente da idade, do GCS admissão e ou a presença de traumas associados. Nenhuma associação foi observada entre os níveis séricos de IL-10 medidos 68 horas após o TCE e a mortalidade (p = 0,22).

Desta forma, pudemos concluir que os níveis séricos de IL-10 medidos no início do 2 primeiros dias após TCE grave estão independentemente associados com mortalidade hospitalar aumentada e pode ser útil como marcador do TCE e seu prognóstico.

ABSTRACT

Traumatic brain injury (TBI) is a worldwide cause of morbidity and mortality. Blood levels of cytokines have been associated with TBI. Here we investigated the serum levels of IL-2, IL-4, IL-5, IL-10, TNF-α and INF-γ as biomarkers of traumatic brain injury severity and its association with hospital mortality.

The serum levels cytokines were determined at a median (IQ 25/75) time of 10 (5/18) hours after TBI in 93 consecutive patients admitted in our hospital. For comparisons, we selected randomly patients with mild (n = 18) and moderate (n = 16) TBI attended in our emergence. In patients with severe TBI 2 additional blood samples were analyzed 30 (22/37) and 68 (55/78) hours after injury. Data collected included age, gender, CT findings, admission GCS and pupillary reactions, presence of associated trauma and hospital mortality.

Even in severe cases, the serum IL2, IL4, IL5 and IFN-γ levels remain undetectable after TBI by flow cytometry. The serum IL-10 levels, but not TNF-α, correlates significantly with GCS severity (Sperman's coefficient = 0.42, $p<0.0001$) and were associated with hospital mortality of patients with severe TBI. The elevated serum IL-10 levels remain significantly associated with mortality ($p = 0.01$) in the subset of patients with isolated severe TBI (n = 74). Multiple logistic regression analysis shows that higher serum IL-10 levels (> 0.09 pg/ml) measured 10 or 30 hours after the injury were respectively 6 times (OR 6.2, CI 95% 1.2 – 25.1, $p = 0.03$) and 5 times (OR 5.4, CI 95% 1.2 – 25.1, $p = 0.03$) more associated with hospital mortality than lower levels (< 0.05 pg/ml) independently on age, admission GCS and pupils or presence of associated trauma. No association was observed between serum IL-10 measured at 68 hour after TBI and mortality ($p = 0.22$).

Serum IL-10 levels measured earlier in the first 2 days after severe TBI are independently associated with higher hospital mortality and may be a useful marker of TBI and its prognosis.

LISTA DE FIGURAS

Figura 1: Correlação entre a GCS na admissão e IL-10 (pg /mL x 10^3), determinada por ELISA, medida 11horas (QI 25/75 = 4-13 horas) após o TCE. Houve uma correlação significativa (p <0,0001) entre o valor de GCS na admissão e IL-10 (correlação de Sperman coeficiente = 0,42) 37

Figura 2: Níves séricos de IL-10 (pg/mL x 10^3), determinada por ELISA medido em pacientes vítimas de TCE grave (sobreviventes e não sobreviventes, TCE moderado e leve. 38

Figura 3 : Correlação entre CSG na admissão e níveis séricos de TNF-α (pg/ml x 10^3), determinados por ELISA, medido 11horas (QI 25/75 = 4-13 horas) após o TCE. Nenhuma correlação significativa foi observada entre a GCS de admissão e as dosagens de TNF-α (coeficiente de correlação de Pearson = - 0,12, p = 0,18). 39

Figure 1A: Correlation between the admission GCS and serum IL-10 (pg/mL x 10^3) determined by ELISA measured in the blood sample collected in a median of 10 hour (IQ 25/75 = 5/18) hours after the TBI. There was a significant correlation (p < 0.0001) between the admission GCS and the serum IL-10 (Sperman's correlation coefficient = 0.42). 71

Figure 1B: Serum levels of IL-10 (pg/mL) determined by ELISA measured in patients with severe (survivors and non-survivors), moderate and mild TBI. The non-survivors patients with severe TBI (n =35) showed a significant enhancements in the serum IL-10, in comparison to survivors (n = 58) and patients with moderate (n = 18) or mild (n = 16) TBI. a = Significant difference (p < 0.001) between patients with severe TBI who survived and patients with mild TBI. b = Significant difference (p < 0.001) between patients with severe TBI who survived and patients with moderate TBI. c = Significant difference (p < 0.02) between survivors patients with severe TBI and those with moderate TBI. d = Significant difference (p < 0.01) between survivors and non-survivors patients. Data expressed in median and Inter-quartile range. The serum IL-10 was determined by ELISA.............................. 72

Figure 1C: Correlation between the admission GCS and serum TNF-α (pg/mL x 10^3) determined by ELISA measured in the blood sample collected in a median of 10 hour (IQ 25/75 = 5/18) hours after the TBI. No significant correlation was observed between the admission GCS and the serum TNF-α (Pearson's correlation coefficient = - 0.12, p = 0.18). The serum TNF-α was determined by ELISA. 73

Figure 1D: Serum levels of TNF-α (pg/mL) determined by ELISA measured 11 (IQ 25/75 = 4/13) hours after the TBI in patients with severe (survivors and non-survivors), moderate and mild TBI. There were no statistical difference in the serum levels of TNF-α among patients with severe (survivors and non-survivors), moderate and mild TBI ($p = 0.18$). Data expressed in median and Inter-quartile range. The serum levels of TNF-α were not correlated with the serum level of IL-10 (Sperman's correlation coefficient = - 0.077, $p = 0.23$)........................ 74

LISTA DE TABELAS

Tabela I: Variáveis clínicas, demográficas, radiológicas e de neurocirurgia e os níveis séricos de IL-10 de acordo com a mortalidade hospitalar. 43

Tabela II: Associação independente entre os níveis séricos de IL-10 medidos no primeiro e segundo dias após o TCE grave e a mortalidade hospitalar. 47

Table 1A: Clinical, demographic, radiological, neurosurgical variables and serum IL-10 levels according to the hospital mortality. 75

Table 1B : Independent association between serum IL-10 levels measured in the first and second days after the severe TBI and hospital mortality. 77

Table 1: Clinical, demographic, radiological, neurosurgical variables and mortality of patients with severe head injury, at the time of discharge. 97

Table 2: Plasma levels of TBARS (nmol/mg protein x 10^{-5}) and protein carbonyl (nmol/mg protein x 10^{-14}) of TBI patients and control group. 99

Table 3: Clinical and laboratorial parameters at the time of the first blood sampling 101

Table 4: Multiple logistic regression model that better explains the association between each categorical variable and death at the time of discharge. 104

LISTA DE ABREVIATURAS

TCE- Traumatismo Crânio Encefálico
SINETRAN- Sindicato Nacional das Empresas de Cursos Especializados para o Trânsito e Transportes
DETRAN- SC- Departamento Estadual de Trânsito de Santa Catarina
TBI- Traumatic Brain Injury
CSG- Escala de Coma de Glasgow
NUPNEC- Núcleo de Pesquisas em Neurologia Experimental e Clínica
UFSC- Universidade Federal de Santa Catarina
SNC- Sistema Nervoso Central
BHE- Barreira Hemato Encefálica
IL- Interleucina
TNF- Fator de Necrose Tumoral
MHC- Complexo de Histocompatibilidade Principal
ROS- Espécies Reativas de Oxigênio
NGF- Fator de Crescimento Neural
KO- Nocaute Genético
LCR- Líquido céfalo-raquidiano (LÍQUOR)
IL-10R1- Receptor de Alta Afinidade da IL-10
IL-10R2- Receptor de Baixa Afinidade IL-10
Tyr43- Tirosina
Phe143- Fenilalanina
TGF-β- Fator de Transformação do Crescimento beta
IFN-γ- Interferon Gama
UTI- Unidade de Terapia ntensiva
$FI0_2$- Fração de oxigênio inspirado
PEEP-Pressão Expiratória Final Positiva
PIC-Pressão Intracraniana
SARA- Síndrome da Angústia Respiratória do Adulto
CBA- Cytometric Bead Array
ELISA- Enzyme-linked Immunosorbent Assay
KPL- Substrato ABTS O-Peroxidase

IC- Intervalo de Confiança
IQ- intervalo inter-quartil
SD- desvio padrão

SUMÁRIO

1. INTRODUÇÃO 23
1.1. O QUE É O TRAUMATISMO CRÂNIO-ENCEFÁLICO E SUA IMPORTÂNCIA MÉDICA-SOCIAL 23
1.2. MEDICINA BASEADA EM EVIDÊNCIA APLICADA AO TCE 23
1.3. EVOLUÇÃO DO DANO CEREBRAL EM PACIENTES VÍTIMAS DE TCE 24
1.4. TNF-ALFA 26
1.5. INTERLEUCINA 10 (IL-10) 28
1.6. JUSTIFICATIVA 29
2.OBJETIVOS 31
2.1 OBJETIVO GERAL 31
2.2 OBJETIVOS ESPECÍFICOS 31
3. MÉTODO 33
3.1. DELINEAMENTO DO ESTUDO 33
3.2. PACIENTES E COLETA DAS AMOSTRAS 33
3.3. ASPECTOS ÉTICOS 33
3.4. VARIÁVEIS DEMOGRÁFICAS, CLÍNICO-LABORATORIAIS, NEUROCIRÚRGICAS E NEUROIMAGEM 33
3.5. NÍVEIS DE INTERLEUCINAS 34
3.6. ANÁLISES ESTATÍSTICAS 35
4. RESULTADOS 37
5. DISCUSSÃO 49
6. REFERÊNCIAS 53
7. APÊNDICES 59
7.1 ARTIGO 1 61
7.2- ARTIGO 2 82
7.3 - ARTIGO 3 105
7.4- ARTIGO 4 107
7.5 - ARTIGO 5 109
7.6 - ARTIGO 6 111

1. INTRODUÇÃO

1.1. O QUE É O TRAUMATISMO CRÂNIO-ENCEFÁLICO E SUA IMPORTÂNCIA MÉDICA-SOCIAL

Trauma crânio-encefálico (TCE) corresponde a qualquer agressão traumática que acarrete lesão anatômica ou comprometimento funcional do couro cabeludo, crânio, meninges ou encéfalo. O traumatismo crânio encefálico (TCE) é a maior causa de morbidade, mortalidade e incapacidade neurológica em adultos jovens (1;2). Em estudos populacionais que incluem todas as idades têm sido observada uma incidência trimodal de maior ocorrência de TCE, que são em crianças menores de um ano, final da adolescência/ início da idade adulta e em idosos (>64 anos). Quanto ao gênero, a maior incidência de TCE é observada em homens, principalmente em adolescentes e adultos jovens (1;2).

O TCE é um problema mundial de saúde pública que já foi denominado "epidemia silenciosa" em função das sequelas que podem não ser prontamente evidentes, do limitado conhecimento científico a respeito das características, extensão e impacto de seus efeitos, e do baixo nível de informação do público sobre o assunto(3). A incidência de TCE em países desenvolvidos é estimada em 1,6 milhões de pessoas a cada ano, aproximadamente 200 por 100.000 habitantes, das quais 270.000 recebem hospitalização.(4) Dessas, 52.000 morrem e 80.000 permanecem com graves sequelas neurológicas, acarretando ao governo um custo direto e indireto estimado de quatro bilhões de dólares ao ano (4).

As principais causas de TCE são acidentes automobilísticos, violência e quedas (2;4). Em Santa Catarina, dados do SINETRAN/DETRAN de 2003, contabilizaram 1679 mortes por acidentes de trânsito, principal causa de TCE, sendo que 81% destas ocorreram em pacientes com idade entre 10 e 50 anos.

1.2. MEDICINA BASEADA EM EVIDÊNCIA APLICADA AO TCE

Tomadas de decisão terapêutica são frequentemente baseadas no prognóstico dos pacientes. Modelos prognósticos são modelos estatísticos que combinam duas ou mais variáveis para predizer o desfecho clínico. Recentemente a “International Mission on Prognosis and Analysis of Clinical Trials in TBI” (IMPACT) publicou uma série de artigos derivados de 8 estudos controlados e 3 observacionais. A

IMPACT demonstrou que a idade, escala de coma de Glasgow, resposta pupilar na admissão e as características tomográficas estão associadas ao prognóstico avaliado 6 meses após o TCE.(2;5-7) Perel e cols. analisaram 31 artigos publicados desde 1990 relacionados ao prognóstico de pacientes com TCE que utilizaram a análise por regressão logística múltipla (8). Um estudo similar foi feito por Mushkudiani e cols.(7). Os autores concluíram que os estudos sobre TCE utilizando modelos prognósticos freqüentemente carecem de: i) uma descrição adequada da coleta e validade das variáveis incluídas no modelo; ii) amostras maiores de casos; iii) adequado manuseio das variáveis e das perdas; iv) mais clareza na apresentação das interações da análise multivariada; v) descrição mais clara das medidas de prognostico; vi) validação externa; vii) clareza na descrição da forma como é feito o modelo. Há também uma carência de estudos em países em desenvolvimento, onde a incidência de TCE é significativamente alta.

O Núcleo de Pesquisas em Neurologia Experimental e Clínica da UFSC (NUPNEC) vêm se dedicando ao estudo do TCE grave. Em 2008 recebemos o aceite de um artigo para publicação da maior série mundial de pacientes com TCE grave (n =748) consecutivos estudada prospectivamente em um único hospital ao longo de 10 anos. (2) A média de idade foi de 35 anos, sendo e 85% do sexo masculino. A mortalidade hospitalar foi de 33%. O estudo confirmou os achados apresentados pela IMPACT e despertou novas linhas a serem abordadas no tema. Em nosso estudo, a capacidade total de predição foi de 76.9%, sendo 87.6% para predição da sobrevida e 55.6% para a predição da mortalidade hospitalar. Uma forma de se aprimorar o modelo prognóstico é identificar marcadores biológicos oriundos de investigação experimental. Esta abordagem também permitirá determinar potenciais alvos terapêuticos que possam minimizar a mortalidade e morbidade associada ao TCE.

1.3. EVOLUÇÃO DO DANO CEREBRAL EM PACIENTES VÍTIMAS DE TCE

Foi apenas nas últimas décadas, que descobrimos que o SNC é capaz de reagir a estímulos imunológicos externos. Até então, o cérebro era considerado "imunologicamente privilegiado", devido à falta de um sistema linfático e pela proteção da barreira hemato-encefálica (BHE) que evitaria a passagem de células e moléculas solúveis. Atualmente, existem evidências para a falha da proteção da barreira hemato-

encefálica, podendo haver transmigração das células do sistema imunológico, em especial os leucócitos após o TCE. A quebra da barreira é na verdade o catalisador para eventos imunológicos dentro do cérebro após traumatismo (9). Durante a fase aguda do trauma, um grande número de monócitos e neutrófilos deixa a corrente sanguínea e migra em direção aos locais onde há lesão tecidual. Neste contexto, sobressaem-se as interleucinas (IL) produzidas pelos macrófagos, linfócitos e leucócitos aderidos ao endotélio, e o fator de necrose tumoral (TNF), sintetizado preferencialmente por macrófagos ativados. Podemos afirmar também que a neuroinflamação após o TCE é também apoiada pelos componentes internos do tecido cerebral. No entanto, os astrócitos e microglia, células residentes no SNC, expressam baixos níveis das classes I e II do Complexo de Histocompatibilidade Principal (MHC), mas também são capazes de sintetizar citocinas, quimiocinas e seus receptores. A capacidade imunológica da glia já foi implicada em uma variedade de patologias do SNC, e seu papel, em qualquer situação, foi mostrado variando de apoptose simples à apresentação de antígenos, resolução de excitotoxicidade à liberação de moléculas neurotóxicas e fatores neurotróficos.

TCE pode ser considerado uma condição neuroinflammatoria do SNC e o trauma cerebral com ruptura da BHE leva ao acúmulo de leucócitos da circulação sistêmica, que iniciam liberação de citocinas pró-inflamatórias, proteases citotóxicas e espécies reativas de oxigênio, por sua vez, iniciam as funções imunológicas da glia(10) . Após o TCE, a microglia torna-se morfológica e imunologicamente indistinguível dos macrófagos infiltrantes da periferia; ambos compartilham da mesma linhagem de monócitos e possuem um papel primordial de defesa através de fagocitose (11,12). Em modelos comumente usados de neuroinflamação, a microglia têm sido caracterizada como sendo a fonte predominante de moléculas pro-inflamatórias, tais como as interleucinas e os espécies reativas de oxigênio (ROS) (13). Em contraste, os astrócitos assumem um papel muito mais favorável, secretando fatores de crescimento, favorecendo a angiogênese, proporcionando fontes alternativas de energia (na forma de glutamina e de lactato) para o recurso empobrecido do neurônio e incentivando a diferenciação dos neurônios recém-formados (14,15). No entanto, nenhum tipo de célula é completamente neurotrófica ou neurotóxica: a microglia têm demonstrado ser fonte de neurotrofinas da família do fator de

crescimento neural (NGF) (16), ao passo que os astrócitos são bem conhecidos por desempenharem um papel na formação da cicatriz glial, um fator de controvérsia na viabilidade dos neurônios sobreviventes e regeneração axonal(17). Essa dualidade é a marca da neuroinflamação no TCE, nenhum fator, molécula ou célula podem ser vistos como puramente negativos ou benéficos na sequência de traumatismo cerebral.

A ativação e proliferação dos astrócitos residentes são típicas de eventos inflamatórios em ambos e difusa no TCE focal (18). No entanto, a influência do sistema imune sobre a resposta cerebral tem sido essencialmente caracterizada por experiências em lesões focais. A seqüência inflamatória de infiltrado neutrofílico inicia no prazo de 24 h do trauma, com atraso de recrutamento de macrófagos em 3-5 dias pós-TCE, essencialmente, aplica-se a contusão no cérebro (19,20). Em contraste, a lesão axonal difusa experimental mostra imunoativação de ambos os astrócitos e microglia e infiltração de macrófagos periféricos, com uma resposta aguda de neutrófilos ausente na circulação sistêmica (21). Essa reação imunológica central diferente pode resultar de vários graus de perturbação da BHE (22) e, além disso, têm uma influência sobre a resposta imune global montada pelo paciente.

Citocinas, produzidas sistemicamente e por via intratecal, uma variedade de moléculas não-imunes e células do sistema imunológico, mediando neuroinflamação: o recrutamento de células hematológicas da periferia, o aumento da permeabilidade vascular cerebral, bem como a ativação continuada de células residentes do SNC estão diretamente envolvidas no processo de inflamação pós TCE (23). Esses mediadores desempenham um papel vital não apenas na resposta de propagação neuroinflamatória, mas também são indicadores úteis de sua presença. Entretanto, as citocinas não são sinônimo de danos; suas funções neuroprotetoras e neurotróficos também são bem caracterizados e são conhecidos por serem necessárias para o neurodesenvolvimento e a manutenção da função normal do SNC (9,24). Assim, as citocinas têm sido os principais exemplos da dicotomia do resposta imune, com a sua detecção formando a base da investigação neuroinflamatória no TCE.

1.4. TNF-ALFA

TNF é considerado como uma das citocinas pró-inflamatórias, no entanto, o seu potencial de propriedades neuroprotetoras começaram a surgir na virada do século. Essa reputação foi obtida principalmente através de sua influência na microglia: TNF é bem conhecido por sua

capacidade de causar aumento da produção e hipertrofia das células do SNC nativas, por sua vez, causando a liberação parácrina de si mesmo a partir dessa fonte celular (25,26). Ao fazê-lo, TNF incentiva o recrutamento de leucócitos da circulação periférica, a liberação de enzimas proteolíticas que levam à degradação do BHE, e inibição de repovoamento astrocitário e regeneração neuronal, especialmente importante na lesão difusa (27,28,29,30).

O TNF tem sido o principal objeto de investigação no domínio da lesão focal, os modelos de lesões focais demonstraram o caráter universal dos efeitos do TNF. Por exemplo, a expressão de TNF foi encontrada no córtex contralateral e hipocampo de ratos feridos com lesões focais. O edema cerebral focal (controlado nos modelos de impacto cortical) foi correlacionado com as alterações microvasculares globais levando ao edema cerebral após a lesão e o inicio do processo inflamatório (24,31,32). Em contrapartida, outros modelos de lesão têm demonstrado a detecção dos níveis séricos de TNF no prazo de 24 h, com ausência de expressão no tecido cerebral, o que dá credibilidade ao argumento de que a lesão difusa provoca uma resposta imune muito diferente(33).

Neutralização de TNF foi realizada através da administração de proteínas de ligação, os receptores solúveis e antagonistas para sua síntese, e todos demonstraram melhora cerebral e atenuação do edema e as disfunções neurológicas(34,35). No entanto, a supressão do TNF na forma de nocaute genético (KO) indicou a dicotomia complexa de ações do TNF. Stahel et al mostrou em 2000 que uma deficiência do gene TNF, em Linfotoxina / TNF-α camundongos KO duplo, resultou em aumento na mortalidade pós-traumática (10). Camundongos TNF-α KO Linfotoxina sobreviventes, apresentaram uma tendência de reforço, recuperação neurológica pós-trauma sem diferença significativa no grau de disfunção da BHE, morte celular e infiltração de neutrófilos, em comparação com animais placebo. Outros modelos de lesão cortical focal apresentaram déficits de memória e diminuição da função motora no período pós-traumático agudo em camundongos KO TNF, enquanto a sua recuperação de uma lesão cortical foi prolongada em quatro semanas e resultou em perda de tecido (36). Essas descobertas instituiram o conceito de dualidade do funcionamento da resposta neuroinflamatoria no TCE: uma citocina que tinha uma reputação forte para causar danos agudos no SNC, agora foi associada com melhor resultado e dano neuronal reduzido no longo prazo. A administração prolongada (quatro semanas) de medicamento anti-inflamatório, o ibuprofeno, resultou em piora na capacidade cognitiva de roedores

submetidos à lesão de percussão fluida, mas não houve diferença significativa na perda de tecido do hipocampo.

TNF elevado em soro e LCR tem sido documentado na clínica de pacientes com TCE grave(37). As propriedades neuroprotetoras do TNF, além de seus efeitos neurotóxicos, têm sido ultimamente reconhecidas no TCE humano, em uma relação inversa com as citocinas IL-18 (pró-inflamatória) e IL-10 (anti-inflamatória) (38). Um estudo de 28 pacientes com TCE, durante um período de 3 semanas, apresentou média de níveis liquóricos de TNF mais elevados que os níveis séricos médios, corroborando com os achados de que há produção endógena de TNF dentro do SNC (38). Um estudo recente, baseado na medição do receptor solúvel de TNF (que têm propriedades anti-inflamatórias, devido à sua capacidade de vincular circulantes de TNF) em 29 pacientes com TCE produzidu o resultado oposto, onde os níveis plasmáticos superaram os do líquor. Isso reforça que, agudamente, fatores pró-inflamatórios prevalecem dentro do SNC, apesar das atividades de mediadores anti-inflamatórios.

1.5. INTERLEUCINA 10 (IL-10)

Interleucina-10 (IL-10) é uma citocina cuja a função primária parece ser a de limitar e controlar as respostas inflamatórias. Ele sinaliza através de interações com duas cadeias do receptor: a de alta afinidade da IL-10R1 e baixa afinidade da IL-10R2. Inicialmente, a IL-10 interage com IL-10R1, formando um complexo intermediário com um sítio de ligação para a cadeia de segundo receptor. Subseqüente ligação da IL-10R2 completa o complexo final. A IL-10 humana é um dímero intercalado por duas subunidades, cada uma composta de 160 resíduos de aminoácidos. Quase 85% dos resíduos de cada subunidade estão envolvidos na formação de seis hélices, designados de A a F. A estrutura do complexo intermediário de IL-10 com um domínio extracelular de IL-10R1 (sIL-10R1) é constituído por uma molécula de IL-10 e duas cópias do receptor ligado a cada domínio do dímero IL-10. A molécula é posicionado sobre o eixo de simetria dupla de modo que ambos os domínios de IL-10 e as metades sIL-10R1 são cópias exatas um do outro. As estruturas de ambos os receptores (livres e ligados) a IL-10 são essencialmente as mesmas moléculas. A maioria das interações do ligante/receptor de interface são de natureza polar, mas com duas manchas hidrofóbicas em torno das cadeias laterais de Tyr43 e Phe143 do receptor. A posição e a estrutura do sítio de ligação para a cadeia de segundo receptor, IL-10R2, ainda são incertas.

A interleucina 10 (IL-10) é um inibidor de macrófagos ativados, o que lhe cabe um importante papel no controle homeostático das reações da imunidade inata e da imunidade celular. Tanto a IL-10 e TGF-β são citocinas anti-inflamatórias com atributos de imunossupressores, e exercem seus efeitos através da inibição de citocinas pró-inflamatórias como TNF-α, IL-1 e IFN-γ (33,39,40,41). Além disso, media ativação glial, inibe a expressão de co-estimuladores e da moléculas do MHC de classe II nos macrófagos, estimula a proliferação de células B humanas em cultura, e atua na inibição da adesão de leucócitos. Estudos experimentais em IL-10 têm demonstrado seus efeitos benéficos, com a administração exógena da citocina auxiliando a recuperação neurológica e redução de citocinas pro- inflamatórias(42). Os níveis de IL-10 no líquor mostram aumento agudo nas primeiras 24 h após TCE, correlacionando com a diminuição da TNF(38,43). Curiosamente, os níveis séricos de IL-10 são elevados tanto em pacientes que sofreram TCE grave, bem como os politraumatizados, tornando-a um marcador não-específico do TCE (44,45). Além disto, a IL-10 tem mostrado correlação com um aumento da mortalidade em pacientes pediátricos, bem como eventos séptico em paciente politraumatizado(46).

1.6. JUSTIFICATIVA

Controlar o grau de resposta inflamatória após uma lesão cerebral pode ser benéfico, pois a inflamação intracraniana pós-traumática tem sido associada com resultados adversos. A fim de elucidar o papel potencial dos mediadores anti-inflamatórios x pró-inflamatórios, a produção de interleucina-10 (IL-10) e TNF-α foi dosada em 3 amostras pareadas de soro de pacientes com traumatismo cranioencefálico grave (TCE) e comparadas em relação a vítimas de TCE leve e moderado.

2.OBJETIVOS

2.1 OBJETIVO GERAL

Investigar a existência de associação entre os níveis de marcadores de resposta inflamatória no sangue venoso periférico que estejam associados ao prognóstico de pacientes com TCE grave.

2.2 OBJETIVOS ESPECÍFICOS

Investigar a existência de associação entre a resposta inflamatória e óbito hospitalar em pacientes com TCE.

Aproximar pesquisadores das áreas básicas e clínica cuja área de atuação envolva trauma;

Formar um pesquisador com base sólida para realizar pesquisa translacional aplicada ao TCE e despertar seu interesse para seguir esta linha de pesquisa com competitividade internacional;

Fortalecer a linha pesquisa em trauma do NUPNEC junto ao Curso de Pós-Graduação em Ciências Médicas da UFSC.

3. MÉTODO

3.1. DELINEAMENTO DO ESTUDO

Estudo de coorte prospectivo

3.2. PACIENTES E COLETA DAS AMOSTRAS

Uma amostra de sangue foi coletada de todos os pacientes o mais rapidamente possível no primeiro dia após a internação hospitalar (sala de emergência ou UTI). Duas amostras adicionais foram coletadas de pacientes com TCE grave de acordo com o cronograma da equipe de pesquisa entre o segundo e o quarto dia após a internação na UTI. As amostras de sangue de pacientes internados no final de semana foram recolhidos nas manhãs de segunda-feira. O intervalo de tempo entre o TCE e a coleta de sangue foi determinado por um dos nossos investigadores com as informações recolhidas junto da ficha de inscrição na emergência e foram altamente precisos. Cinco mililitros de sangue venoso periférico foram coletados e centrifugados. O soro foi armazenado por 24h a -20 ° C e depois a -70 ° C até a análise imunológica.

3.3. ASPECTOS ÉTICOS

Este estudo integra outro projeto, já em andamento, denominado “NEUROTRAUMA: BASES FISIOPATOLÓGICAS, DIAGNÓSTICO, FATORES PROGNÓSTICOS E TRATAMENTO”, registrado na CONEP e aprovado pelo Comitê de Ética em Pesquisas da UFSC.

3.4. VARIÁVEIS DEMOGRÁFICAS, CLÍNICO-LABORATORIAIS, NEUROCIRÚRGICAS E NEUROIMAGEM

As variáveis a serem coletadas durante a interação do paciente são:

a) Na admissão, em 24, 48 e 72 horas após a admissão: Escala de coma de Glasgow, Sinais vitais, Exame pupilar, Gasometria arterial, Hemograma, Glicemia, Sódio, Potássio, Uréia, Creatinina, Função hepática. No caso de ventilação mecânica: Freqüência respiratória, Fração de oxigênio inspirado ($FI0_2$), Pressão Expiratória Final Positiva (PEEP).

b) Na admissão e durante a internação: nome, idade, sexo, estado civil, escolaridade, renda familiar, Histórico documentado de doença psiquiátrica, data e hora do TCE, data e hora de admissão em UTI, traumatismos associados, ressucitação hemodinâmina, Tomografia computadorizada na admissão (escala de Marshall), tipo de lesão intra-craniana, reposição de volume na admissão, transfusão sangüínea, monitorização de pressão intracraniana (PIC), traqueostomia, coma induzido, administração de drogas vasoativas, oximetria do bulbo jugular, Infecção respiratória, Infecção urinária, septicemia, síndrome da angústia respiratória do Adulto (SARA).

c) No momento da alta os pacientes sobreviventes serão classificados de acordo com a "escala de "*Outcome* de Glasgow" (CSG).

3.5. NÍVEIS DE INTERLEUCINAS

Para investigarmos a associação entre os níveis séricos de citocinas e a gravidade do TCE em relação ao tempo após a lesão, realizamos uma triagem inicial da única amostra de sangue coletadas de pacientes leves (n = 11), moderados (n = 11) e na primeira amostra coletadas de pacientes com TCE grave (n = 11) . A escolha das amostras foi realizada de modo randomizado, cego para todas as variáveis de internação, exceto a gravidade do trauma.

A fim de investigar uma possível associação entre os níveis de citocinas e o tempo após a lesão provocada pelo TCE, também medimos, por citometria de fluxo, as amostras dos pacientes. No soro, medimos IL-2, IL-4, IL-5, IL-10, IFN-γ e TNF-α nas amostras de sangue coletadas no primeiro, segundo e terceiro dias de pacientes vítimas de TCE grave (n = 9 pacientes / grupo). A escolha das amostras foi realizada de modo randomizado, cego para todas as variáveis de internação, exceto a pontuação CGS admissão.

Um citômetro de fluxo (FACSSCalibur, BD Biosciences, EUA) foi utilizado para determinação sérica de citocinas usando o BD TM Cytometric Bead Array (CBA) Humano de citocinas Th1/Th2 (BD Biosciences, San Diego, CA). O kit CBA empregado permite a discriminação das seguintes citocinas: IL-2 (menor nível dedetecção de 2,6 pg / mL), IL-4 (menor nível de detecção de 2,6 pg / mL), IL-5 (menor nível de detecção de 2,4 pg / mL), IL-10 (menor detecção do

nível 2,8 pg / mL), IFN-γ (nível inferior de detecção 7,1 pg/ml e TNF-α (nível inferior de detecção 2,8 pg / mL) em uma única amostra. O processamento das amostras e a análise de dados foram realizadas de acordo com as instruções do fabricante. Resumidamente, as amostras de soro foram incubadas com 6 beads de citocinas de captura e anticorpos de detecção PE-conjugados durante 3 h, à temperatura ambiente e protegidas da luz. Posteriormente, as amostras foram lavadas e os dados foram obtidos por meio do citômetro de fluxo FACSScalibur (BD Biosciences, San Diego, CA). Os resultados das amostras foram gerados em formato gráfico e tabular usando o GraphPad Prism versão 5.01.

Devido ao fato de que apenas IL-10 e TNF-α foram detectados no primeiro dia após TCE, foram medidos os níveis séricos dessas citocinas em maior número de casos. Utilizamos então pacientes leves (n = 16), moderados (n = 18) e graves (n = 93) para novas detecções realizadas por ELISA (Enzyme-linked Immunosorbent Assay), utilizando-se kits comerciais de ELISA (BD OptEIA ™ELISA Kit, TNF-α, e BD OptEIA ™ELISA Kit para IL-10. Basicamente, as placas de 96 poços (Immulon 2HB de TNF-α e Maxisorp NUNC para IL-10) foram revestidas com anticorpo monoclonal específico para cada citocina. Padrões e amostras foram adicionados aos poços, e qualquer IL-10 e TNF-α presentes, ligaram-se ao anticorpo imobilizado. Os poços foram lavados e a streptavidina peroxidase misturada com o anticorpo biotinilado anti IL-10 e TNF-α -humano foram adicionados, produzindo um complexo anticorpo-antígeno-anticorpo foramando um "sanduíche", o princípio empregado por este método. Os poços foram novamente lavados e o substrato ABTS O-Peroxidase (KPL) foi então adicionado, o que produz uma cor azul em proporção direta com a quantidade de citocinas presentes na amostra inicial. A solução utilizada para parar a ligação foi SDS 10% e a absorbância foi lida a 405 nm.

3.6. ANÁLISES ESTATÍSTICAS

As análises estatísticas foram realizadas, utilizando o programa de estatística SPSS 12.0. Foram realizadas análises univariadas para estabelecer o grau de associação entre as variáveis dependentes e as independentes. As variáveis dependentes analisadas foram: a mortalidade e a escala de “outcome de glasgow” no momento da alta. As variáveis independentes avaliadas foram: as variáveis clínico-

demográficas, laboratoriais, suporte hemodinâmico e ventilatório, neuroimagem, marcadores de estresse oxidativo, marcadores imunológicos, neurocirúrgicas e demais descritas na metodologia.

Realizamos uma análise univariada para determinar a associação entre a presença das variáveis dependentes e cada uma das variáveis independentes em estudo. Nesta análise as variáveis categóricas foram analisadas através de teste exato de Fisher ou qui-quadrado (dependendo da distribuição quantitativa das variáveis). Variáveis contínuas foram analisadas através de teste “t” de Student, Mann-Whitney, Kruskal-Wallis (seguidos de pós-hoc), dependendo do número de grupos estudados e da existência ou não de normalidade, determinada através do teste de Kolmogorow-Smirnov. A medida de associação entre a variável dependente (presença de um diagnóstico psiquiátrico) e cada uma das variáveis independentes foi medida através do “crude odds ratio” e o respectivo intervalo de confiança de 95%.

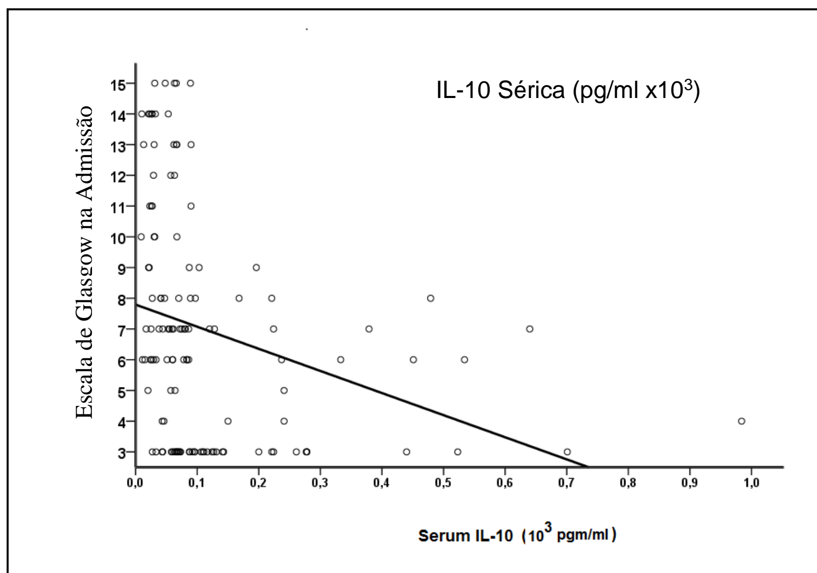
Num segundo momento, através de uma regressão logística binária, determinamos o grau de associação entre as variáveis dependentes (descritas acima) e as variáveis independentes cuja análise univariada mostrou uma associação com nível de significância de “p” < 0.20. Então, determinamos o modelo de regressão logística que melhor explicou a associação entre as variáveis clínico-demográficas, laboratoriais, suporte hemodinâmico e ventilatório, neuroimagem, níveis de marcadores imunológicos, neurocirúrgicas e demais descritas na metodologia e as variáveis independentes investigadas.

Após as análises finais, os níveis de “p” inferiores a 0.01 foram considerados estatisticamente significativos. Este maior rigor para os níveis aceitáveis de significância foram estabelecidos para minimizar a possibilidade de erro do tipo 1 devido a realização de múltiplas comparações (47).

4. RESULTADOS

As amostras de sangue foram coletadas em um tempo médio (IQ intervalo 25-75) de 10 horas (05-18h) para a primeira coleta, 30horas (22-37h) para a segunda coleta e 68 horas (55-78h) para a terceira coleta após o TCE. Na primeira amostra de sangue de pacientes com TCE leve, moderado e grave (n = 10/grupo) os níveis séricos de IL-2, IL-4, IL-5 e INF-γ foram indetectáveis pela técnica do CBA (dados não mostrados).Porque IL-10 e TNF-α foram detectados nessa triagem inicial, estas citocinas foram determinados em todos os pacientes com TCE leve(n = 16), moderado (n = 18) e grave (n = 93) (ver figura 1). Não houve diferença no tempo de coleta de sangue entre os três grupos (Kruskal-Wallis, p = 0,23, dados não mostrados). A IL-10 tem os níveis aumentados com a gravidade do TCE determinada pela CSG (Figura 1A).

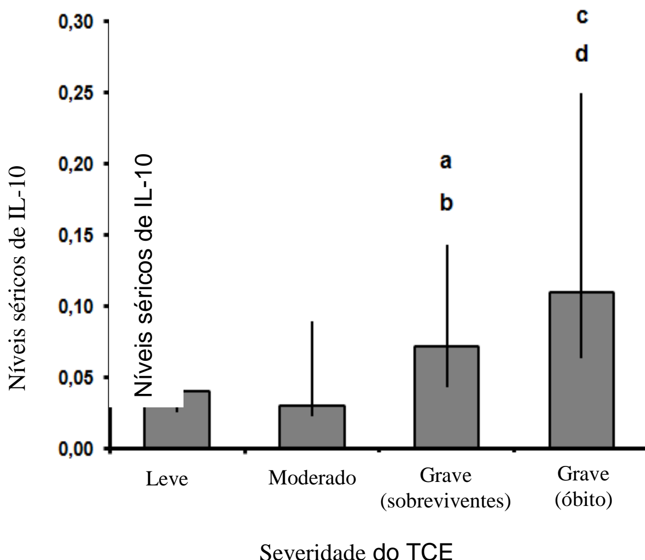
Figura 1: Correlação entre a GCS na admissão e IL-10 (pg /mL x 10^3), determinada por ELISA, medida 11horas (QI 25/75 = 4-13 horas) após o TCE. Houve uma correlação significativa (p <0,0001) entre o valor de GCS na admissão e IL-10 (correlação de Sperman coeficiente = 0,42)



Fonte:SPSS

Figura 2 mostra que os níveis séricos de IL-10 foram significativamente maiores em não sobreviventes do que os sobreviventes com TCE grave.

Figura 2: Níves séricos de IL-10 (pg/mL x 10^3), determinada por ELISA medido em pacientes vítimas de TCE grave (sobreviventes e não sobreviventes, TCE moderado e leve.

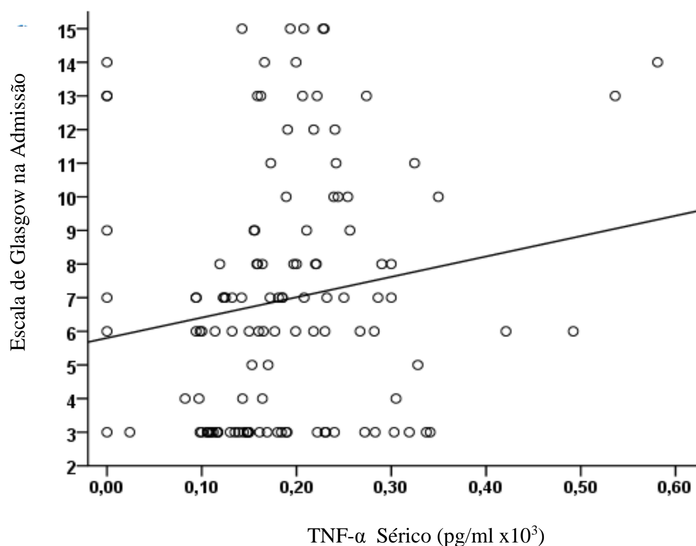


Fonte:SPSS

Os pacientes não sobreviventes com TCE grave (n = 35) apresentaram melhoras significativas no soro de IL-10, em relação aos sobreviventes (n = 58) e pacientes com (n = 18) ou moderada (n = 16) leve TCE. a = diferença significativa (p <0,001) entre pacientes com TCE grave que sobreviveram e os pacientes com TCE leve. b = diferença significativa (p <0,001) entre pacientes com TCE grave que sobreviveram e os pacientes com TCE moderado. c = diferença significativa (p <0,02) entre os pacientes sobreviventes com trauma grave e aqueles com TCE moderado. d = diferença significativa (p <0,01) entre sobreviventes e não sobreviventes. Dados expressos em mediana e intervalo inter-quartil. A IL-10 foi determinada por ELISA.

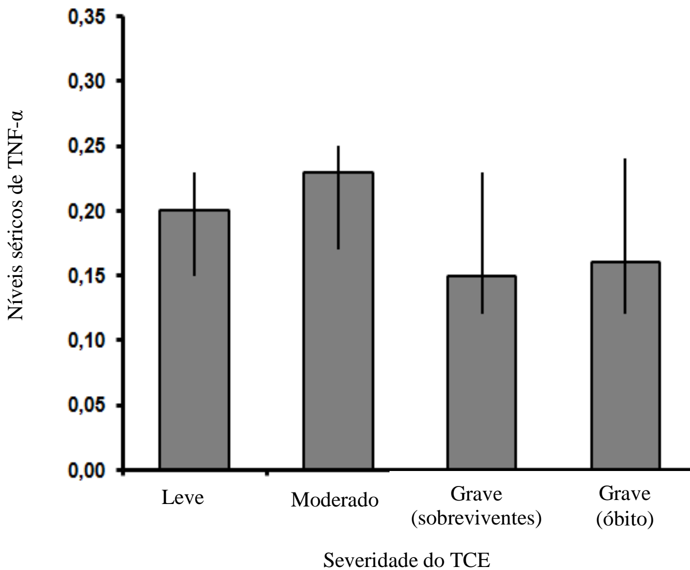
A Figura 3A mostra que os níveis de TNF-α não foram correlacionados com a gravidade do trauma medido pela CSG e não foram associados com a mortalidade (Figura 3 B).

Figura 3 : Correlação entre CSG na admissão e níveis séricos de TNF-α (pg/ml x 10^3), determinados por ELISA, medido 11horas (QI 25/75 = 4-13 horas) após o TCE. Nenhuma correlação significativa foi observada entre a GCS de admissão e as dosagens de TNF-α (coeficiente de correlação de Pearson = - 0,12, p = 0,18).



Fonte:SPSS

Figura 4: Os níveis séricos de TNF-α (pg / mL), determinada por ELISA, medidos 11 horas (QI 25/75 = 4-13 horas) após o TCE em pacientes graves (sobreviventes e não sobreviventes), moderados e leves. Não houve diferença estatística nos níveis séricos de TNF-α entre os pacientes com casos graves (sobreviventes e não sobreviventes), moderados e leves ($p = 0,18$). Dados expressos em mediana e intervalo inter-quartil. Os níveis séricos de TNF-α não foram correlacionados com o nível sérico de IL-10 (coeficiente de correlação de Sperman = - 0,077, $p = 0,23$).



Fonte:SPSS

A análise da associação entre níveis séricos de IL-10 e mortalidade hospitalar em pacientes com TCE grave isolado foram realizados na primeira ($n = 74$) e na segunda amostra ($n = 60$) de sangue. Pacientes com TCE grave isolado que morreram apresentavam nível sérico significativamente maior de IL-10 do que os sobreviventes. Na primeira amostra, os níveis de IL-10 (mediana de IQ 25-75) dos sobreviventes foi de 70pg/ml (IQ 40-110pg/ml) em comparação com 120pg/ml (IQ 60-260pg/ml) nos não-sobreviventes ($p = 0,01$). Na segunda amostra de sangue, os níveis de IL-10 de sobreviventes foi de 60pg/ml (IQ 40 – 80pg/ml) em comparação a 90pg/ml (IQ 50-

140pg/ml) em não sobreviventes ($p = 0,02$). Não houve diferença ($p=1,0$) entre a IL-10 medida na terceira amostra de sangue coletada quando comparamos sobreviventes e não sobreviventes com TCE grave isolado (dados não mostrados). Os níveis séricos deTNF-α na primeira, segunda e terceira amostras de sangue também foram semelhantes ($p> 0,29$) entre sobreviventes e não sobreviventes com TCE grave isolado.

Os níveis de IL-2, IL-4, IL-5 e INF-γ foram indetectáveis pelo CBA (dados não mostrados) na primeira (mediana de 12 horas, QI 25/75 intervalo variando entre 8-20 horas), segunda(mediana de 34 horas, QI 25/75 intervalo variando entre 23-37 horas) e a terceira amostra (mediana de 65 horas, QI 25/75 intervalo 56-79 horas) coletada após o TCE grave ($n = 9$ pacientes/cada tempo). A IL-10 e TNF-α são mostrados na tabela I.

Todos os pacientes com TCE leve sobreviveram e um paciente com TCE moderado morreu. As características clínicas, demográficas, radiológicos, variáveis de neurocirurgia, IL-10 e os níveis de mortalidade hospitalar de pacientes com TCE grave são apresentados na Tabela I. A idade média dos pacientes foi de 35 anos e 82% eram do sexo masculino. Trinta e sete por cento ($n = 35$) morreram durante a hospitalização. A causa da morte foi TCE para 32 pacientes. Um paciente morreu devido a infarto do miocárdio, outro devido a uma pneumonia, derrame pleural e parada cardíaca e outro por pneumonia com evolução para sepse, insuficiência renal e parada cardíaca. Estes três pacientes morreram após a terceira coleta de amostras de sangue. A mortalidade hospitalar não esteve associada ao sexo, os achados da TC de admissão e traumas associados ($p \geq 0,29$). Havia uma tendência não significativa para uma maior mortalidade dos pacientes com idade superior a 44 anos ($p = 0,13$). CSG de admissão inferior a 5 foi de cinco vezes mais associado à mortalidade hospitalar do que em pacientes que apresentavam maior escore de CSG (ORbruto 4,4, IC 95%, 1,5 - 13, $p = 0,007$). A presença de anormalidades nas pupilas na admissão foi de quase 6 vezes maior nos não-sobreviventes que nos sobreviventes (OR bruto de 5,9, IC 95% 2,3-15,4, $p <0,001$). A análise univariada mostrou uma associação entre maiores níveis de IL-10 e mortalidade hospitalar. Os níveis séricos de IL-10 maior que 90 pg / mL na primeira amostra de sangue foi de 10 vezes mais associado à morte do que a IL-10 níveis inferiores a 50 pg / ml (bruto ou 10, IC 95% 2,0 - 48,9 p, = 0,004). A IL-10 níveis entre 50 e 90 pg /ml na primeira amostra foram seis vezes mais associados à morte (OR 6,2, IC 95% 1,2-31,1, $p = 0,03$). Quanto à segunda amostra de sangue, houve uma tendência não

significativa para a associação entre os níveis séricos de IL-10 níveis maior que 90 pg / ml e de mortalidade (brutas ou 2,7, IC 95% 0,8-8,9, p = 0,10) em comparação com os níveis menores. Os níveis séricos de IL-10, medidos na terceira amostra de sangue não foi associado com a mortalidade hospitalar ($p \geq 0,22$). Todas essas análises permanecem inalteradas quando as mortes não excluindo atribuída a morte encefálica (dados não mostrados). O tempo após o TCE e os 3 tempos de coleta de sangue não diferem entre sobreviventes e não sobreviventes (p> 0,22, ver tabela I).

Tabela I: Variáveis clínicas, demográficas, radiológicas e de neurocirurgia e os níveis séricos de IL-10 de acordo com a mortalidade hospitalar.

Variáveis	Outcome			Crude OR Para Óbito (95 CI)	*p* Value
	Todos os Pacientes *n* = 93 (%)	Sobreviventes *n* = 58 (%)	Não-sobreviventes *n* = 35 (%)		
Idade					
Média (± SD)	35.4 (15.7)	34.0 (15.9)	37.5 (17.0)	N.A.	
18 a 44 anos	67 (72.0)	45 (77.6)	22 (62.9)	1.0	
Mais de 44 anos	26 (28.0)	13 (22.4)	13 (37.1)	2.0 (0.8 – 5.1)	0.13
Genero					
Masculino	77 (82.8)	49 (84.5)	28 (80.0)	1.0	
Feminino	16 (17.2)	09 (15.5)	07 (20.0)	1.4 (0.5 – 4.0)	0.58
Marshall CT [a]					
Lesão Tipo I					
Lesão Tipo II	04 (4.3)	03 (5.2)	01 (2.9)	1.0	
Lesão Tipo III	22 (23.7)	16 (27.6)	06 (17.1)	1.1 (0.1 – 13.0)	0.92
Lesão Tipo IV	14 (15.1)	09 (15.5)	05 (14.3)	1.7 (0.1 – 20.5)	0.69
Evacuated mass lesion	07 (7.5)	05 (8.6)	02 (5.7)	1.2 (0.1 – 19.6)	0.90
Non-evacuated lesion	21 (22.6)	13 (22.4)	08 (22.9)	1.8 (0.2 – 20.9)	0.62
	22 (23.7)	10 (17.2)	12 (34.3)	3.6 (0.3 – 40.2)	0.29
SAH [a]					
Não	39 (41.9)	25 (43.9)	14 (41.2)	1.0	
Sim	52 (55.9)	32 (56.1)	20 (58.8)	1.10 (0.6 – 2.7)	0.82
Trauma Associado					
Sim	32 (34.4)	21 (36.2)	11 (31.4)	1.0	
Não	61 (65.6)	37 (63.8)	24 (68.6)	1.2 (0.5 – 3.0)	0.64
GCS na Admissão					
7 or 8	28 (30.1)	22 (37.9)	06 (17.1)	1.0	
5 or 6	21 (22.6)	16 (27.6)	05 (14.3)	1.1 (0.3 – 4.4)	0.85
3 or 4	44 (47.3)	20 (34.5)	24 (68.6)	4.4 (1.5 – 13.0)	0.007
Pupilas na Admissão					
Isocorica	45 (48.4)	37 (63.8)	08 (22.9)	1.0	
Anormal	48 (51.6)	21 (36.2)	27 (77.1)	5.9 (2.3 – 15.4)	< 0.001
Níveis séricos de citocinas na 1ª amostra	n = 93	n = 58	n = 35		

Variáveis	Todos os Pacientes n = 93 (%)	Outcome: Sobreviventes n = 58 (%)	Outcome: Não-sobreviventes n = 35 (%)	Crude OR Para Óbito (95 CI)	p Value
Horas após TCE (IQ 25-75)	10 (5 - 18)	10 (5 – 22)	10 (4 – 15)	N.A.	0.22
Serum IL-10 levels, pg/ml (IQ 25-75)	0.08 (0.05 - 0.16) [d]	0.07 (0.04 - 0.12)	0.11 (0.06 – 0.24)	N.A.	< 0.0001
IL-10 < 50	21 (22.6)	19 (32.2)	02 (5.7)	1.0	
IL-10 ≥ 50 ≤ 90	33 (35.5)	21 (35.6)	13 (37.1)	6.2 (1.2 – 31.1)	0.03
Serum TNF-α, pg/ml (IQ 25-75)	0.16 (0.12 – 0.23)	0.16 (0.12 – 0.23)	0.16 (0.12 – 0.20)	N.A.	0.78
Serum cytokines in the 2nd sample [b]	n = 76	n = 47	n = 29		
Hours after TBI (IQ 25-75)	30 (22 – 37) [e]	30 (21 – 37)	27 (22 – 35)	N.A.	0.37
Níveis de IL-10, pg/ml (IQ 25-75)	0.07 (0.04 – 0.11)	0.07 (0.04 - 0.09)	0.09 (0.05 – 0.15)	N.A.	0.02
IL-10 < 50	23 (30.3)	16 (34.0)	07 (24.1)	1.0	
IL-10 ≥ 50 ≤ 90	29 (31.2)	20 (42.6)	09 (25.7)	1.0 (0.3 – 0.4)	0.96
IL-10 > 90	24 (25.8)	11 (23.4)	13 (44.8)	2.7 (0.8 – 8.9)	0.10
TNF-α Sérico, pg/ml (IQ 25-75)	0.21 (0.18 – 0.25) [f]	0.21 (0.19 – 0.26)	0.21 (0.17 – 0.23)	N.A.	0.55
IL-10 sérica na 3ª amostra	n = 44	n = 31	n = 13		
Horas após o TCE (IQ 25-75)	68 (55 – 78)	71 (61 – 80)	67 (54 – 76)	N.A.	
Níveis de IL-10 , pg/ml (IQ 25-75)	0.04 (0.03 – 0.07)	0.04 (0.03 – 0.06)	0.06 (0.03 -0.10)	N.A.	0.28
IL-10 < 50	25 (26.9)	19 (61.3)	06 (46.2)	1.0	
IL-10 ≥ 50 ≤ 90	13 (29.5)	09 (29.0)	04 (30.8)	1.4 (0.3 – 6.2)	0.65
Il-10 > 90	06 (13.6)	03 (09.7)	03 (23.1)	3.2 (0.5 – 20.0)	0.22

a- tomografia computadorizada não foi analisada em 2 casos; b- Níveis séricos de IL-10 não foram examinados nas segunda amostra de sangue em 17 pacientes. Sete pacientes morreram antes da hora de amostragem, e em onze segunda amostra não foram recolhidas pela equipe de pesquisa; c Níveis séricos de IL-10 não foram analisados no terceiro dia em 49 pacientes. Em seguida, morreu antes da época de amostragem e em 39 asegunda amostra não foi recolhida pela equipe de pesquisa. d Níveis séricos de IL-10 apresentaram reduções significativas nas segunda e terceiras amostras em comparação à primeira amostra (≤ 0,005). e níveis séricos de IL-10 na terceira amostra foram menores do que na segunda amostra de sangue (p <0,05). f Houve uma melhora significativa no nível de TNF-α da segunda amostra em comparação à primeira amostra de sangue. TNF-α não foi mensurado na terceira amostra de sangue.

Finalmente, a análise de regressão logística múltipla é mostrada na tabela II. A presença de níveis mais altos de IL-10 (> 90 pg / ml) na primeira ou na segunda amostra de sangue foram, respectivamente, 6 vezes (OR ajustado 6,2, IC 95% 1,2-25,1, p = 0,03) e 5 vezes (OR ajustado 5,4, IC 95% 1,2-25,1, p = 0,03) mais associados com a mortalidade do que os níveis mais baixos (<50 pg / ml), independentemente da idade, do GCS admissão e alunos ou a presença de traumas associados (ver tabela II). Os níveis séricos IL10 entre 50 e 90 pg /ml medidos na primeira amostra de sangue revela uma tendência não significativa de associação independente com a morte em comparação com os níveis mais baixos (OR ajustado4,8, IC 95%, 0,8-27,7, p = 0,08) . Nós incluímos a variável "trauma associado" no modelo final da análise de regressão logística múltipla que demonstrou que a associação observada entre a concentração sérica de IL-10 e a mortalidade não foi devido ao viés de confundimento relacionado com a presença de lesões em outros órgãos.

O modelo de regressão incluindo a CSG, o exame, os traumas associados e os níveis de IL-10 na primeira coleta de sangue mostraram 73% de predição correta global com a sobrevivência e a morte anunciada de 86% e 51%respectivamente. Quando os níveis séricos de IL-10 da segunda coleta de amostras de sangue foram incluidos no lugar da primeira amostra de sangue, o modelo de regressão mostrou 71% de predição correta global com a sobrevivência e a morte anunciadade 77% e 60% respectivamente. Os níveis de IL-10 na terceira amostra de sangue, não foram incluídos na análise de regressão, pois elevado número de amostras faltavam e também porque não estava associada com o prognóstico na análise univariada.

Tabela II: Associação independente entre os níveis séricos de IL-10 medidos no primeiro e segundo dias após o TCE grave e a mortalidade hospitalar.

Variáveis	Outcome: Todos Pacientes n = 93 (%)	Sobreviventes n = 58 (%)	Não-sobreviventes n = 35 (%)	OR [a] Para Morte (95% IC)	p Valor	OR [b] Para Morte (95% IC)	p Valor
Idade em anos							
18 a 44 anos	67 (72.0)	45 (77.6)	22 (62.9)	1.0		1.0	
Mais de 44 anos	26 (28.0)	13 (22.4)	13 (37.1)	1.9 (0.7 – 5.5)	0.23	2.8 (0.8 – 9.3)	0.09
Trauma Associado							
Sim	32 (34.4)	21 (36.2)	11 (31.4)	1.0		1.0	
Não	61 (65.6)	37 (63.8)	24 (68.6)	1.2 (0.4 – 3.9)	0.80	1.4 (0.4 – 5.5)	0.60
CSG da Admissão							
5 ou maior	45 (48.10)	35 (60.3)	10 (28.6)	1.0		1.0	
Menor do que 5	48 (51.6)	23 (39.7)	25 (71.4)	2.85 (1.1 -8.0)	0.04	4.6 (1.4 – 15.6)	0.01
Pupilas na Admissão							
Isocorica	45 (48.4)	37 (63.8)	08 (22.9)	1.0		1.0	
Anormal	48 (51.6)	21 (36.2)	27 (77.1)	4.9 (1,7 – 14.1)	0.003	7.6 (2.1 – 28.0)	0.002
IL-10 Sérica na 1ª amostra de sangue	(n = 93)	n = 58	n = 35				
IL-10 < 0.05	21 (22.6)	19 (32.2)	02 (5.7)				
IL-10 ≥ 0.05 ≤ 0.09	33 (35.5)	21 (35.6)	13 (37.1)	4.8 (0.84 – 27.7)	0.08	N.A.	N.A.
IL-10 > 0.09	39 (41.9)	19 (32.2)	20 (57.1)	6.2 (1.1 – 34.3)	0.03	N.A.	N.A.
IL-10 Sérica na 2ª amostra de sangue	(n = 76)	n = 47	n = 29				
IL-10 < 0.05	23 (30.3)	16 (34.0)	07 (24.1)	N.A.	N.A.	1.0	
IL-10 ≥ 0.05 ≤ 0.09	29 (31.2)	20 (42.6)	09 (25.7)	N.A.	N.A.	2.1 (0.5 – 9.2)	0.33
IL-10 > 0.09	24 (25.8)	11 (23.4)	13 (44.8)	N.A.	N.A.	5.4 (1.2 – 25.1)	0.03

a OR ajustado para idade, traumas associados, CSG de admissão, pupilas de admissão e níveis de IL-10 medidos na primeira amostra de sangue coletada. b OR ajustado para idade, traumas associados, CSG de admissão e níveis de IL-10 medidos na segunda amostra de sangue coletada. IL-10 não foi examinado no segundo dia em 17 pacientes, pois sete pacientes morreram antes do tempo de amostragem e em onze a segunda amostra de sangue não foi colhido.

5. DISCUSSÃO

O conceito de dualidade de neuroinflamação no TCE é agora um aspecto bem reconhecido da pesquisa de traumas. As conclusões que podem ser obtidas na literatura até o momento, em grande parte, giram em torno da noção de que a resposta imune do SNC é dicotômica, em suas múltiplas facetas: quer seja a reação pró- versus anti-inflamatória, como na lesão focal versus difusa, a resposta central versus resposta periférica, ou quadro agudo em relação ao quadro crônico. O SNC já não é visto como uma entidade resistente à infiltração imune periférica, mas sim influenciado pela imunoativação central que pode promover eventos imunológicos periféricos. Na inflamação aguda, os mecanismos neuroreparativos aparecem semanas a meses após a lesão. Sabemos, agora, que seria demasiado simplista inibir qualquer mediador inflamatório farmacológicamente em qualquer momento e esperar uma resposta previsível. Assim, o impulso à plena elucidação da patogenia do TCE é mais forte do que nunca. Para produzir as opções de tratamento viável para o paciente vítima de TCE, a importância de compreendermos a resposta imune, celular e suas interações, não pode ser subestimada.

Inicialmente, esperávamos encontrar uma resposta de citocinas pró-inflamatórias mais acentuada. No entanto, nossos dados revelaram que a IL-10 encontrada em níveis elevados nas primeiras horas após o TCE está intrinsicamente ligada ao maior número de óbitos. Dessas forma, corroborando com outros achados (42,44) podemos supor que a IL-10 diminui neuroinflamação central enquanto, causa imunossupressão no paciente perifericamente. Isto é especialmente importante nos pacientes politraumatizados que envolvem resposta sistêmica e podem estar contribuindo para a lesão cerebral secundária (por exemplo, aumentando a suscetibilidade à infecção), apesar do fato de que as citocinas anti-inflamatórias reduzirem danos cerebrais secundários mediados por neuroinflamação. Outra particularidade da interação entre eventos imunológicos intracerebrais e periféricos é que o aumento da produção de citocinas central pode render um melhor resultado de reposta local, mas a produção periférica pode agravar o resultado global.

O presente estudo demonstrou que os níveis de IL-10, mas não os níveis de TNF-α, correlacionam-se com CSG da admissão e estão independentemente associados com a mortalidade hospitalar em pacientes com TCE grave. Como já demonstrado em outras populações, o aumento inicial de IL-10 após o TCE evolui para níveis mais baixos

nos primeiros três dias após a lesão (REF 17, 24, 25). A associação entre a concentração sérica de IL-10 e os níveis de mortalidade por TCE foi demonstrada anteriormente por Bell et al.em crianças (46) e adultos com lesão corporal, incluindo alguns pacientes semTCE (45), mas estes dados não foram confirmados por Dziurdzik et al. em pacientes adultos com TCE (49). Nós acreditamos que o tamanho pequeno da amostra de pacientes desses estudos anteriores justifica suas conclusões contraditórias. O observado aumento nos níveis de IL-10 pode ser devido a uma lesão extra-craniana adicional (45), a liberação monocítica rápida após a ativação simpática no TCE isolado (18) ou até mesmo produzido em altas concentrações pela microglia residente e infiltração de monócitos/macrófagos na fase aguda da lesão (14). O último mecanismo também pode contribuir com a elevação dos níveis séricos de TNF-α (14). Diferentemente dos níveis de IL-10, em nosso estudo, o padrão dos níveis séricos de TNF-α aumenta nos primeiros dois dias após o TCE grave, mas seus níveis não foram associados com a gravidade do TCE medido pela CSG bem como a mortalidade hospitalar em pacientes vítimas de TCE grave.

Acreditamos que a associação entre os níveis séricos elevados de IL-10 medidos até 48 horas após o TCE grave e mortalidade hospitalar em nossos pacientes não é espúrio, porque: 1) o tamanho amostral de pacientes foi adequado para testar a hipótese, 2) os desequilíbrios da distribução das variáveis entre os sobreviventes e não sobreviventes provou-se independentemente, associados com o prognóstico de TCE grave foram controlados por análise de regressão; 3) existe uma plausibilidade biológica para os níveis mais elevados da citocina IL-10 ser neuroprotetor em casos mais graves, 4) os níveis inalterados de IL-2, IL-4, IL-5 e INF-γ medidos em 30 amostras de sangue de pacientes a reduziu a possibilidade de mudanças em níveis de citocinas, devido à reação inespecífica a lesão. O desenho do estudo atual não nos permite chegar a uma conclusão se os níveis elevados de IL-10 em não sobreviventes é protetor, deletério ou apenas um marcador de outro mecanismo reconhecido, relacionado com o prognóstico do paciente. Curiosamente, Knoblache faden em 1998 mostrou que a administração sistêmica (intravenosa ou subcutânea), mas não a administração intracerebroventricular de IL-10, apresentou uma melhora neurológica em um modelo de percussão fluido-lateral do TCE (ref 28), sugerindoque a IL-10 poderia ser não só um marcador prognóstico, mas também um alvo terapêutico para o tratamento do TCE.

Se confirmados em outras populações, nossos achados indicam que a IL-10 pode ser um marcador promissor do TCE e seu prognóstico.

A associação entre os níveis séricos de IL-10 e ambos os resultados imediatos e a longo prazo avaliados por habilidades motoras, cognitivas e avaliação psiquiátrica, bem como aspectos da qualidade de vida são pontos importantes a serem investigados em pacientes vítimas de TCE.

6. REFERÊNCIAS

(1) Marion DW SMT-KEC. Craniocerebral Trauma. In: Bradley WG DRFGJJ, ed. Neurology in clinical practice, Fourth ed. Philadelphia: Butterworth Heinemann, 2004:1127-1147.

(2) Martins ET, Linhares MN, Sousa DS, et al. Mortality in severe traumatic brain injury: a multivariated analysis of 748 brazilian patients from Florianópolis city. *J Trauma* 2008;in press.

(3) Goldstein M. Traumatic brain injury: a silent epidemic. *Ann Neurol* 1990 Mar;27(3):327.

(4) Marion DW, Shartz MC, Tyler-Kabara EC. Trauma of the Nervous System. Craniocerebral Trauma. In: Bradley WG, Daroff RB, Fenichel GM, Jankovic J, eds. Neurology in Clinical Practice, Fourth ed. Philadelphia: Buterworth Heinemann, 2004:1127-1147.

(5) McHugh GS, Engel DC, Butcher I, et al. Prognostic value of secondary insults in traumatic brain injury: results from the IMPACT study. *J Neurotrauma* 2007 Feb;24(2):287-293.

(6) Murray GD, Butcher I, McHugh GS, et al. Multivariable prognostic analysis in traumatic brain injury: results from the IMPACT study. *J Neurotrauma* 2007 Feb;24(2):329-337.

(7) Mushkudiani NA, Hukkelhoven CW, Hernandez AV, et al. A systematic review finds methodological improvements necessary for prognostic models in determining traumatic brain injury outcomes. *J Clin Epidemiol* 2008 Apr;61(4):331-343.

(8) Perel P, Edwards P, Wentz R, Roberts I. Systematic review of prognostic models in traumatic brain injury. *BMC Med Inform Decis Mak* 2006;6:38.

(9) Benveniste EN. Cytokine actions in the central nervous system. *Cytokine Growth Factor Rev*. 1998;9(3–4):259–275.

(10) Stahel PF, Shohami E, Younis FM, et al. Experimental closed head injury: analysis of neurological outcome, blood-brain barrier dysfunction, intracranial neutrophil infiltration, and neuronal cell death in mice deficient in genes for pro-inflammatory cytokines. *J Cereb Blood Flow Metab*. 2000;20(2):369–380.

(11) Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: intrinsic immuneffector cell of the brain. *Brain Res Brain Res Rev*. 1995;20(3):269–287.

(12)Raivich G, Bohatschek M, Kloss CU, et al. Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. *Brain Res Brain Res Rev*. 1999;30(1):77–105.

(13) Nakajima K, Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. *Curr Drug Targets Cardiovasc Haematol Disord*. 2004;4(1):65–84.

(14) Liberto CM, Albrecht PJ, Herx LM, et al. Pro-regenerative properties of cytokine-activated astrocytes. *J Neurochem*. 2004;89(5):1092–1100.

(15) Marion DW. Lactate and traumatic brain injury. *Crit Care Med*. 1999;27(9):2063–2064.

(16) Elkabes S, DiCicco-Bloom EM, Black IB. Brain microglia / macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J Neurosci*. 1996;16(8):2508–2521.

(17) Rostworowski M, Balasingam V, Chabot S, et al. Astrogliosis in the neonatal and adult murine brain post-trauma: elevation of inflammatory cytokines and the lack of requirement for endogenous interferon-gamma. *J Neurosci*. 1997;17(10):3664–3674.

(18) Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T. Inflammatory response in acute traumatic brain injury: a double-edged sword. *Curr Opin Crit Care*. 2002;8(2):101–105.

(19) Clark RS, Schiding JK, Kaczorowski SL, et al. Neutrophil accumulation after traumatic brain injury in rats: comparison of weight drop and controlled cortical impact models. *J Neurotrauma*. 1994;11(5):499–506.

(20) Holmin S, Soderlund J, Biberfeld P, Mathiesen T. Intracerebral inflammation after human brain contusion. *Neurosurgery*. 1998;42(2):291–298 [discussion 298-9].

(21) Csuka E, Hans VH, Ammann E, et al. Cell activation and inflammatory response following traumatic axonal injury in the rat. *Neuroreport*. 2000;11(11):2587–2590.

(22) Barzo P, Marmarou A, Fatouros P, et al. Magnetic resonance imaging-monitored acute blood-brain barrier changes in experimental traumatic brain injury. *J Neurosurg*. 1996;85(6):1113–1121.

(23) Lucas S, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. *Br J Pharmacol*. 2006;147(S1):232–240.

(24) Maier B, Laurer HL, Rose S, et al. Physiological levels of pro- and anti-inflammatory mediators in cerebrospinal fluid and plasma: a normative study. *J Neurotrauma*. 2005;22(7):822–835.

(25) Kita T, Liu L, Tanaka N, Kinoshita Y. The expression of tumor necrosis factor-alpha in the rat brain after fluid percussive injury. *Int J Legal Med*. 1997;110(6):305–311.

(26) Shohami E, Ginis I, Hallenbeck JM. Dual role of tumor necrosis factor alpha in brain injury. *Cytokine Growth Factor Rev*. 1999;10(2):119–130.

(27) Faber-Elman A, Lavie V, Schvartz I, et al. Vitronectin overrides a negative effect of TNF-alpha on astrocyte migration. *Faseb J*. 1995;9(15):1605–1613.

(28) Pober JS, Cotran RS. Cytokines and endothelial cell biology. *Physiol Rev*. 1990;70(2):427–451

(29) Rosenberg GA, Estrada EY, Dencoff JE, Stetler-Stevenson WG. Tumor necrosis factor-alpha-induced gelatinase B causes delayed opening of the blood-brain barrier: an expanded therapeutic window. *Brain Res*. 1995;703(1–2):151–155.

(30) Tchelingerian JL, Monge M, Le Saux F, et al. Differential oligodendroglial expression of the tumor necrosis factor receptors in vivo and in vitro. *J Neurochem*. 1995;65(5):2377–2380.

(31) Fan L, Young PR, Barone FC, et al. Experimental brain injury induces differential expression of tumor necrosis factor-alpha mRNA in the CNS. *Brain Res Mol Brain Res*.1996;36(2):287–291.

(32) Taupin V, Toulmond S, Serrano A, et al. Increase in IL-6, IL-1 and TNF levels in rat brain following traumatic lesion. Influence of pre- and post-traumatic treatment with Ro5 4864, a peripheral-type (p site) benzodiazepine ligand. *J Neuroimmunol*. 1993;42(2):177–185

(33) Kamm K, Vanderkolk W, Lawrence C, et al. The effect of traumatic brain injury upon the concentration and expression of

interleukin-1beta and interleukin-10 in the rat. *J Trauma*.2006;60(1):152–157.

(34) Knoblach SM, Fan L, Faden AI. Early neuronal expression of tumor necrosis factor-alpha after experimental brain injury contributes to neurological impairment. *J Neuroimmunol*.1999;95(1–2):115–125.

(35) Shohami E, Bass R, Wallach D, et al. Inhibition of tumor necrosis factor alpha (TNFalpha) activity in rat brain is associated with cerebroprotection after closed head injury. *J Cereb Blood Flow Metab*. 1996;16(3):378–384.

(36) Scherbel U, Raghupathi R, Nakamura M, et al. Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. *Proc Natl Acad Sci USA*.1999;96(15):8721–8726.

(37) Goodman JC, Robertson CS, Grossman RG, Narayan RK. Elevation of tumor necrosis factor in head injury. *J Neuroimmunol*. 1990;30(2–3):213–217.

(38) Csuka E, Morganti-Kossmann MC, Lenzlinger PM, et al. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. *J Neuroimmunol*. 1999;101(2):211–221

(39) Benveniste EN, Tang LP, Law RM. Differential regulation of astrocyte TNF-alpha expression by the cytokines TGF-beta, IL-6 and IL-10. *Int J Dev Neurosci*. 1995;13(3–4):341–349.

(40) Kremlev SG, Palmer C. Interleukin-10 inhibits endotoxin-induced pro-inflammatory cytokines in microglial cell cultures. *J Neuroimmunol*. 2005;162(1–2):71–80.

(41) Morganti-Kossmann MC, Rancan M, Otto VI, et al. Role of cerebral inflammation after traumatic brain injury: a revisited concept. *Shock*. 2001;16(3):165–177.

(42) Knoblach SM, Faden AI. Interleukin-10 improves outcome and alters proinflammatory cytokine expression after experimental traumatic brain injury. *Exp Neurol*. 1998;153(1):143–151.

(43) Morganti-Kossmann MC, Hans VH, Lenzlinger PM, et al. TGF-beta is elevated in the CSF of patients with severe traumatic brain injuries and parallels blood-brain barrier function. *J Neurotrauma*. 1999;16(7):617–628.

(44) Shimonkevitz R, Bar-Or D, Harris L, et al. Transient monocyte release of interleukin-10 in response to traumatic brain injury. *Shock*. 1999;12(1):10–16.

(45) Shiozaki T, Hayakata T, Tasaki O, et al. Cerebrospinal fluid concentrations of anti-inflammatory mediators in early-phase severe traumatic brain injury. *Shock*. 2005;23(5):406–410.

(46) Bell MJ, Kochanek PM, Doughty LA, et al. Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. *J Neurotrauma*. 1997;14(7):451–457.

(47) Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998 Apr 18;316(7139):1236-38

(48) Neidhardt R, Keel M, Steckholzer U, et al. Relationship of interleukin-10 plasma levels to severity of injury and clinical outcome in injured patients. *J Trauma*. 1997;42(5):863–870[discussion 870-1

(49) Dziurdzik P, Krawczyk L, Jalowiecki P, Kondera-Anasz Z and Menon LSerum interleukin-10 in ICU patients with severe acute central nervous system injuriesInflammation Research . 2004. Vol 53: 338-343.

7. APÊNDICES

Os apêndices serão apresentados na forma de artigos científicos realizados durante o período do mestrado.

7.1 ARTIGO 1

Situação: submetido a Neuroimmunomodulation
Faz parte da Avaliação para a obtenção do título de mestre.

Serum IL-10 enhancement is associated with traumatic brain injury severity and is a independent marker of hospital mortality in severe traumatic brain injury patients

Flavia Mahatma Schneider Soares[1], Nicole Menezes de Souza[2], Marcelo Libório Schwarzbold[1], Alexandre Paim Diaz[1], Alexandre Hohl[1], Priscilla Nunes Abreu da Silva[1], Juliana Vieira[1], Jean Carlos[1], Melina Moré Bertotti[3], Rui Prediger[4], Bafica A[2], Linhares MN [1,3,5], Walz R[1,6].

1. Centro de Neurociências Aplicadas (CeNAp), Hospital Universitário (HU), Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brasil.
2. Laboratório de Imunologia e Doenças Infecciosas (LIDI), Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brasil.
3. Serviço de Neurocirurgia, Hospital Governador Celso Ramos (HGCR), Florianópolis, Brasil.
4. Laboratório de Doenças Neurodegenerativas, Departamento de Farmacologia, Florianópolis, Brasil.
5. Departamento de Cirurgia, HU, UFSC, Florianópolis, Brasil.
6. Departamento de Clínica Médica, HU, UFSC, Florianópolis, Brasil.

Adress for correspondence: Departamento de Clínica Médica, Hospital Universitário, UFSC, 3 andar, Campus Universitário, Trindade, Florianópolis, SC, Brazil. E-mail: rogerwalz@hotmail.com

Background: Traumatic brain injury (TBI) is a worldwide cause of morbidity and mortality. Blood levels of cytokines have been associated with TBI. Here we investigated the serum levels of IL-2, IL-4, IL-5, IL-10, TNF-α and INF-γ as biomarkers of traumatic brain injury severity and its association with hospital mortality.

Methods: The serum levels cytokines were determined at a median (IQ 25/75) time of 10 (5/18) hours after TBI in 93 consecutive patients admitted in our hospital. For comparisons, we selected randomly patients with mild (n = 18) and moderate (n = 16) TBI attended in our emergence. In patients with severe TBI 2 additional blood samples were analyzed 30 (22/37) and 68 (55/78) hours after injury. Data collected included age, gender, CT findings, admission GCS and pupillary reactions, presence of associated trauma and hospital mortality.

Results: Even in severe cases, the serum IL2, IL4, IL5 and IFN-γ levels remain undetectable after TBI by flow cytometry. The serum IL-10 levels, but not TNF-α, correlates significantly with GCS severity (Sperman's coefficient = 0.42, $p<0.0001$) and were associated with hospital mortality of patients with severe TBI. The elevated serum IL-10 levels remain significantly associated with mortality ($p = 0.01$) in the subset of patients with isolated severe TBI (n = 74). Multiple logistic regression analysis shows that higher serum IL-10 levels (> 0.09 pg/ml) measured 10 or 30 hours after the injury were respectively 6 times (OR 6.2, CI 95% 1.2 – 25.1, $p = 0.03$) and 5 times (OR 5.4, CI 95% 1.2 – 25.1, $p = 0.03$) more associated with hospital mortality than lower levels (< 0.05 pg/ml) independently on age, admission GCS and pupils or presence of associated trauma. No association was observed between serum IL-10 measured at 68 hours after TBI and hospital mortality ($p = 0.22$).

Conclusions: Serum IL-10 levels measured in the first 2 days after severe TBI are independently associated with hospital mortality and may be a useful marker of TBI and its prognosis.

INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of mortality and disability among young adults and constitutes a major health and socioeconomic problem throughout the world (1-3). The extent of brain damage is determined by the severity of primary mechanical injury and intensity of secondary biomolecular injury cascades causing neuroinflammation that contributes to cerebral edema, enhanced intracranial pressure and delayed cellular destruction (3,4). Traumatic brain injury results in an early inflammatory response that is initiated by the release of numerous immune mediators followed by the infiltration and accumulation of polymorphonuclear leukocytes and they guide a sequence of events including expression of adhesion molecules, cellular infiltration, and additional secretion of inflammatory molecules and growth factors, resulting in either regeneration or cell death (5-10).

Cytokines are critical mediators of neuroinflammation after TBI (6,9) regulating a wide-variety of cellular functions through autocrine and paracrine signaling networks that initiate and leaving the body in a vicious cycle of hyperinflammation (10). Increases in the synthesis and release of various pro/antiinflammatory cytokines into CNS and blood is associated with severe TBI (5). In animal models, early induction of TNFα and IL-1β that peaks within 3-8h of injury, followed by more sustained elevations of IL-6 and IL-10 (6, 15,18). TNFα and IL-10 are produced in high concentrations by resident microglia and infiltrating monocyte/macrophages in the acute phase of injury (14). Astrocyte reactivity is stimulated by inflammatory cytokines, contributing to increased neuroinflammation and development of secondary injury following neurotrauma (16).

Association studies in human TBI may help to identify biomarkers of prognosis as well targets to develop of treatment strategies. Several studies demonstrated the association between the cerebrospinal fluid or blood levels of different cytokines and human TBI, but their relative small sample sizes of patients, selection bias, inadequate handling of missing data and the confounding bias leaves doubt about the independent association between cytokines and patients prognosis. Here we investigated the independent association between serial serum cytokine levels collected after severe TBI and the hospital mortality of patients using a multiple logistic regression analysis.

Patients and Methods

We included prospectively 93 consecutive adult patients (age 18 to 79 years) with severe TBI (GCS = 3-8) admitted to the intensive care unit of the Hospital Governador Celso Ramos between April 2006 and september 2008 and met the inclusion criteria. The neurosurgical team, the intensive care unit staff, and the research team were the same along this study. The inclusion criteria were Coma Glasgow scale (GCS) score 8 or lower at the hospital admission. Victims of gunshot injury and patients who evolved to brain death before 24 hours of admission were excluded. For comparisons, we also selected randomly patients with mild (GCS = 13-15, n = 18) and moderate (GCS = 9-12, n = 16) TBI attended in our emergence room during the same period and accepted to participate in the study. Informed consent was obtained from patients or their families in more severe cases. No patients had previous neurological disease.

The demographic and hospital variables analyzed were age, gender, computed tomography (CT) findings according the Marshal CT classification [19] and presence of subarachnoid hemorrhage), presence of associated trauma (thorax, abdomen or limbs), CGS and pupil examination at admission. A blind CT analysis was done before the patient's discharge by one of the researchers (MMB) and confirmed by another (MNL), when necessary. Most of these isolated or combined variables were proven to be associated with TBI outcome in our and other populations (1-3, 19-23). The outcome analyzed was death during hospitalization.

A blood sample was collected from all the included patients in the first day after the hospital admission (emergence room or ICU). Two additional samples were collected from patients with severe TBI according to the research team schedule between the second and the fourth day after the ICU admission. The blood samples of patients admitted on weekends were collected on Monday mornings. The time span between the TBI and blood sampling was determined by one of our investigators using the information collected from the rescue registration form and was highly accurate. Five milliliters of peripheral venous blood were collected and centrifuged. The plasma was stored for 24h at -20 ^{o}C and then at -70 ^{o}C until the immunological analysis.

To investigate the association between the serum cytokine levels and TBI severity at earlier time after the injury we performed an initial screening in the single blood sample collected from patients with mild, moderate, and the first sample collected from patients with severe TBI (n = 11 patients/group). The serum sample choice of our serum bank was carried out in a randomized manner blinded for all the hospitalization variables except the severity of trauma. The cytokine analysis was done in a blind manner for the hospitalization variables and outcome of patients. A flow cytometer (FACSCalibur, BD Biosciences, USA) was used for serum cytokines determination using the BD ™ Cytometric Bead Array (CBA) Human Th1/Th2 Cytokine Kit (BD Biosciences, San Diego, CA). The CBA kit employed her allows for the discrimination of the following cytokines: IL2 and IL4 (lower detection level 2.6 pg/mL), IL5 (lower detection level 2.4 pg/mL), IL10 and TNF-α (lower detection level 2.8 pg/mL), and IFN-γ (lower detection level 7.1 pg/mL) in the same single sample processed and analyzed according to the manufacturer's instructions. Briefly, serum samples were incubated with the six cytokine capture beads and PE-conjugated detection antibodies for 3h, at room temperature and protected from light. Afterward, samples were washed and sample data were acquired using FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). Sample results were generated in graphical and tabular format using the GraphPad Prism 5.01

Because only IL-10 and TNF-α were detectable in the first day after the TBI, we measured the serum level of those cytokines in a higher number of cases with mild (n = 16), moderate (n = 18) and severe (n = 93) patients by Enzyme-linked Immunosorbent Assay (ELISA), using commercial ELISA kits (BD OptEIA™ ELISA Kit II for TNF-α and ELISA Ready-SET-Go! Kit for IL-10). Basically, the 96-well plates (Immulon 2HB for TNF-α and NUNC Maxisorp for IL-10) were coated with monoclonal antibody specific for each cytokine. Standards and samples are added to the wells, and any IL-10 and TNF-α present binds to the immobilized antibody. The wells are washed and streptavidin-

horseradish peroxidase conjugate mixed with biotinylated anti-human IL-10 and TNF-α antibody is added, producing an antibody-antigen-antibody “sandwich”, principle of method. The wells are again washed and ABTSÒPeroxidase substrate (KPL) solution is added, which produces a blue color in direct proportion to the amount of cytokines present in the initial sample. The Stop Solution used was SDS 10% and the absorbance is read at 405 nm.

In order to investigate a possible association between the cytokines levels and TBI in later times after the injury we also measured by flow cytometry the serum IL2, IL4, IL5, IL10, IFN-γ and TNF-α in the second and third blood sample collected of patients with severe TBI (n = 9 patients/group). The blood sample choice from the serum database for the initial screening was carried out in a randomized manner blinded for all the hospitalization variables except the severity of trauma and time of sample collection (first, second or third sample). Because only IL-10 and TNF-α were detectable in the second and third sample we also investigated these cytokines in the samples of remain patients with severe TBI.

Statistical Analysis

The continuous variables normality was determined by the One-Sample Kolmogorov-Smirnov test. The correlation between serum cytokines and the severity of trauma (GCS score) were analyzed by Spearman’s (for non-parametric data) or Pearson’s (for parametric data) correlation. Because the asymmetric sample size of groups we used a non-parametric analysis (Kruskal-Wallis followed by Mann-Whitney test) to analyze differences in the cytokine levels among patients with mild, moderate and severe (survivors and non-survivors) TBI. Differences among serum cytokine levels of the three blood samples collected from patients with severe TBI were analyzed by Wilcoxon signed ranks test.

We also analyzed the outcome of patients and the hospital mortality was the dependent variable and the demographic, clinical, radiological, neurosurgical and laboratorial parameters were the independent variables. The cytokine levels were also considered independent variables. Continuous variables were analyzed by Mann-

Whitney test because of the relative asymmetry in the sample size of the survivors and non-survivors groups. Categorical variables were analyzed by binary logistic regression. The magnitude of the association between death and the independent categorical variables was measured by the odds ratio (OR) and respective 95% confidence interval (CI) estimated by unconditional logistic regression.

To identify variables that were independently associated with death, a multiple logistic regression was performed using the following conditional method. For this analysis, continuous variables including the cytokine levels were also categorized. Variables showing associations with death through the univariate analysis with a "p" level of significance lower than 0.20 were included in the analysis. The variable "associated trauma" was also included in the multivariate analysis to exclude the possible confounding bias affecting the cytokine levels due to multiple organs trauma. The probability of stepwise entry was 0.05 and removal 0.10. The classification cutoff was 0.5 with maximum interactions of 20. The "p" levels lower than 0.05 were considered significant. Because the biologic plausibility and to avoid type II error we did not adjust for multiple tests using a more stringent criterion for the "p" level [24]. To minimize the possible bias caused by the presence of multiple organ traumas, we also performed a separated analysis of association between serum levels of IL-10 and the hospital mortality of patients with isolated severe TBI only. Missing cases occurred by chance and were clearly described in the results. Statistical analysis was done using the SPSS program 10.0 (Chicago, IL).

RESULTS

The blood samples were collected in a median (IQ range 25-75) time of 10 (5-18), 30 (22–37) and 68 (55–78) hours after the TBI. In the first blood sample of patients with mild, moderate and severe TBI (n = 10/group) the serum IL2, IL4. IL5 and INF-γ levels were undetectable by CBA (data not shown). Because serum IL-10 and TNF-α levels were detected in this initial screening, these cytokine were determined in all patients with mild (n = 16), moderate (n = 18), severe (n = 93) TBI (see figure 1). There were no differences in the time between the TBI and blood sample collection among the 3 studied groups (Kruskal-Wallis, p = 0.23, data not shown). The serum IL-10 levels increases with the TBI severity determined by GCS (See Figure 1A). The Figure 1B shows that serum levels of IL-10 were significantly higher in non-survivors than survivors with severe TBI. The Figure 1C showed that TNF-α levels

were not correlated with severity of trauma measured by GCS and were not associated with mortality (see Figure 1D).

The separated analysis of the association between serum levels of IL-10 and hospital mortality in patients with isolated severe TBI were carried out in the first (n = 74) and the second (n = 60) blood sample. Patients with isolated severe TBI who died showed a significant higher serum level of IL-10 than survivors. In the first sample, the serum IL-10 levels (median, IQ 25-75) of survivors was 0.07 (IQ 0.04 – 0.11) pg/ml in comparison to 0.12 (IQ 0.06 – 0.26) in the non-survivors (p = 0.01). In the second blood sample, the serum IL-10 levels of survivors was 0.06 (IQ 0.04 – 0.08) in comparison to 0.09 (IQ 0.05 – 0.14) in non-survivors (p = 0.02). There were no differences (p = 1.0) between the serum IL-10 measured in the third blood sample collected of survivors and non-survivors patients with isolated severe TBI (data not shown). Serum levels of TNF-α in the first, second and third blood sample were also similar (p > 0.29) between survivors and non-survivors patients with isolated severe TBI.

The serum IL2, IL4. IL5 and INF-γ levels were undetectable by CBA (data not shown) in the first (Median 12 hours, IQ 25/75 range 8 - 20 hours), second (median 34, IQ 25/75 range 23 - 37 hours), and third (Median 65, IQ 25/75 range 56 - 79 hours) sample collected after the severe TBI (n = 9 patients/time). The serum IL-10 and TNF-α are showed in the table I.

All patients with mild TBI survived and one patient with moderate TBI died. The clinical, demographic, radiological, neurosurgical variables, serum IL-10 levels and hospital mortality of patients with severe TBI are showed in the Table I. The mean age of patients was 35 years and 82% were male. Thirty seven percent (n = 35) died during hospitalization. The death cause was TBI itself for 32 patients. One patient died due to heart infarction, one due to pneumonia, pleural effusion and cardiac arrest and one due to pneumonia evolving to sepsis, renal failure and cardiac arrest. These 3 patients died after the third blood sample collection. Hospital mortality was not associated with gender, admission CT findings and associated trauma (p ≥ 0.29). There was a non-significant trend for a higher mortality of patients older than 44 years (p = 0.13). The admission GCS lower than 5 was five times more associated with hospital mortality than higher GCS scores (crude OR 4.4, CI 95%, 1.5 – 13, p = 0.007). The presence of admission pupilary abnormalities was almost 6 times greater in non-survivors than in survivors (crude OR 5.9,

CI 95% 2.3 – 15.4, $p < 0.001$). The univariated analysis showed an association between higer IL-10 levels and hospital mortality. The serum levels of IL-10 higher than 0.09 pg/mL in the first blood sample was 10 times more associated with death than IL-10 levels lower than 0.05 pg/ml (crude OR 10, CI 95% 2.0 – 48.9, $p = 0.004$). The serum IL-10 levels between 0.05 and 0.09 pg/ml in the first sample were 6 times more associated with death (crude OR 6.2, CI 95% 1.2 – 31.1, $p = 0.03$). Concerning the second blood sample, there was a non-significant trend for association between serum IL-10 levels higher than 0.09 pg/ml and mortality (crude OR 2.7, CI 95% 0.8 – 8.9, $p = 0.10$) in comparison to the lower levels. The serum levels of IL-10 measured in the third blood sample was not associated with hospital mortality ($p \geq 0.22$). All those analyses remain unaltered when excluding deaths not attributed to brain death (data not shown). The time after TBI and the 3 blood sample collection does not differ between survivors and non-survivors ($p > 0.22$, see table I).

Finally, the multiple logistic regression analysis is showed in the table II. The presence of higher levels of serum IL-10 (> 0.09 pg/ml) in the first or in the second blood sample were respectively 6 times (adjusted OR 6.2, CI 95% 1.2 – 25.1, $p = 0.03$) and 5 times (adjusted OR 5.4, CI 95% 1.2 – 25.1, $p = 0.03$) more associated with hospital mortality than lower levels (< 0.05 pg/ml) independently on age, admission GCS and pupils or presence of associated trauma (see table II). The serum IL10 levels between 0.05 and 0.09 pg/ml measured in the first blood sample shows a non-significant trend of independent association with death in comparison to lower levels (adjusted OR 4.8, CI 95%, 0.8 – 27.7, $p = 0.08$). We included the variable “associated trauma” in the final model of multiple logistic regression analysis to demonstrated that observed association between higher serum IL-10 and mortality was not due to confound bias related to presence of other organs lesions. For reasons of analysis power we decide not include in the same multiple logistic regression model the serum IL-10 categories of the first and second blood samples.

The regression model including CGS, pupils examination, associated trauma and IL-10 levels in the first blood collection showed 73% of overall correct prediction with the survival and death predicted at 86% and 51% respectively. When IL-10 serum levels of the second blood sample collection was include in place of the first blood sample, the regression model showed a 71% of overall correct prediction with the survival and death predicted at 77% and 60% respectively. The IL-10 levels in the third blood sample were not included in any regression

analysis because the elevated number of sample missing and because it was not associated with prognosis in the univariate analysis.

DISCUSSION

The present demonstrates that serum IL-10 levels, but not TNF-α, correlates with admission GCS and is independently associated with hospital mortality in patients with severe TBI. As previously demonstrated in other populations, the initial rise of IL-10 earlier times after the TBI evolve to low levels in the first three days after injury (17, 24, 25). The association between higher serum IL-10 levels and mortality due to TBI was demonstrated previously by Bell et al. in children (25) and adults with body injury including some patients without TBI (26) but not confirmed by Dziurdzik et al. in adult patients with TBI (24). We believe the small sample size of patients of these previous studies justify their contradictory findings. The observed serum IL-10 enhancement may be due to additional extra-cranial injury (27), rapid monocytic release after sympathetic activation in isolated TBI (18) or even produced in high concentrations by resident microglia and infiltrating monocyte/macrophages in the acute phase of injury (ref 14). The last mechanism also may contribute with the elevation of TNF-α serum levels (ref 14). Differently than IL-10, in our study the pattern of TNF-α serum level enhances in the first 2 days after the severe TBI, but its levels were not associated with the TBI severity measured by GCS as well the hospital mortality in severe TBI patients.

We believe that the association between the elevated serum IL-10 measured up to 48 hours after the severe TBI and hospital mortality in our patients is not spurious because: 1) the adequate sample size of patients to test the hypothesis; 2) imbalances between survivors and non-survivors distribution of variables that has been proved to be independently associated with the prognosis of severe TBI were controlled by the regression analysis; 3) 4) there is a biological plausibility for the higher levels of the neuroprotective cytokine IL-10 in more severe cases; 5) the unaltered levels of IL-2, IL-4, IL-5, and INF-γ measured in 30 blood samples of patients reduce the possibility of changes in cytokines levels due to unspecific reaction to injury. The present study design does not allow us to reach any conclusion if serum IL-10 enhancement in non-survivors is protective, deleterious or only a marker of another unrecognized mechanism related to the patient prognosis. Interestingly, Knoblach and Faden in 1998 showed that systemic (intravenous or subcutaneous) but not intracerebroventricular

administration of IL-10 improved neurological outcome in a lateral fluid-percussion model of TBI (ref 28), suggesting that IL-10 pathway could be not only a prognostic marker but also a therapeutic target for TBI treatment.

If confirmed in other populations, our findings indicate that IL-10 may be a promising marker of TBI and its prognosis. The association among serum levels of IL-10 and both early and long-term outcomes evaluated by fine motor skills, cognitive and psychiatric evaluation as well as quality of life aspects are important points to be investigated in patients with TBI.

Figure 1A**:** Correlation between the admission GCS and serum IL-10 (pg/mL x 10^3) determined by ELISA measured in the blood sample collected in a median of 10 hour (IQ 25/75 = 5/18) hours after the TBI. There was a significant correlation ($p < 0.0001$) between the admission GCS and the serum IL-10 (Sperman's correlation coefficient = 0.42).

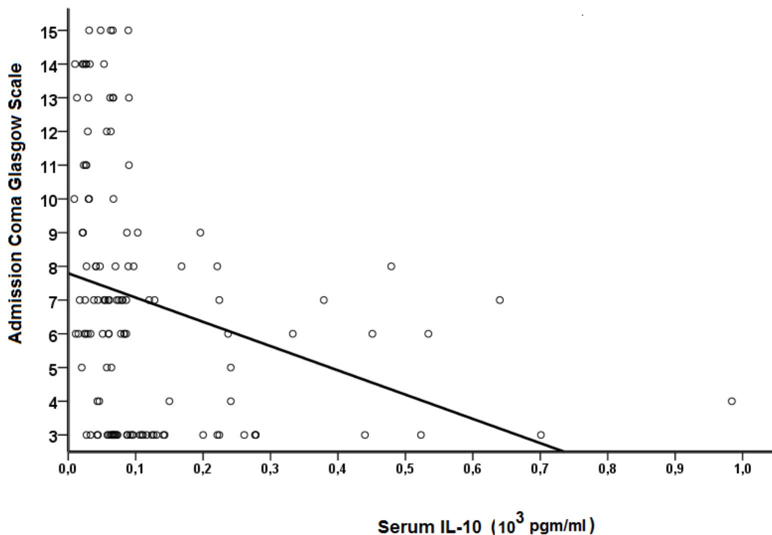


Figure 1B: Serum levels of IL-10 (pg/mL) determined by ELISA measured in patients with severe (survivors and non-survivors), moderate and mild TBI. The non-survivors patients with severe TBI (n =35) showed a significant enhancements in the serum IL-10, in comparison to survivors (n = 58) and patients with moderate (n = 18) or mild (n = 16) TBI. a = Significant difference ($p < 0.001$) between patients with severe TBI who survived and patients with mild TBI. b = Significant difference ($p < 0.001$) between patients with severe TBI who survived and patients with moderate TBI. c = Significant difference ($p < 0.02$) between survivors patients with severe TBI and those with moderate TBI. d = Significant difference ($p < 0.01$) between survivors and non-survivors patients. Data expressed in median and Inter-quartile range. The serum IL-10 was determined by ELISA.

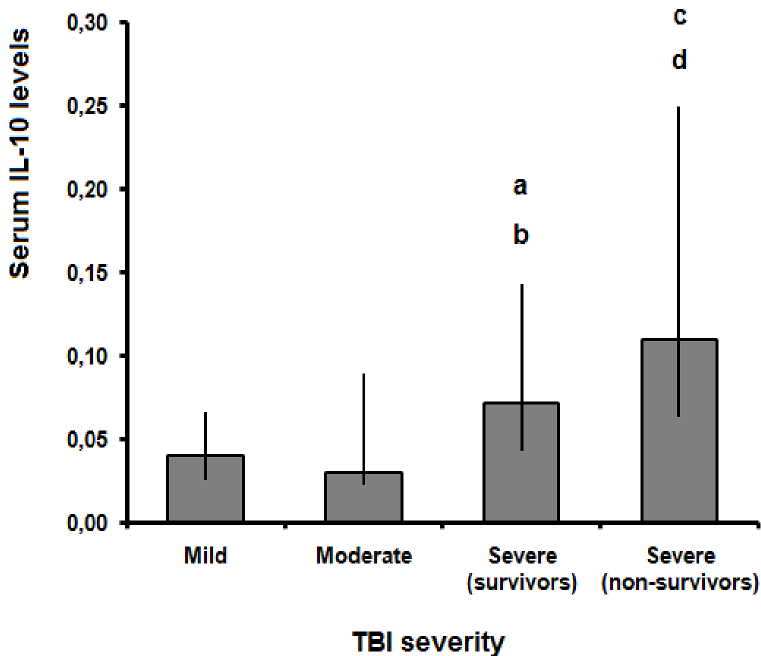


Figure 1C: Correlation between the admission GCS and serum TNF-α (pg/mL x 10^3) determined by ELISA measured in the blood sample collected in a median of 10 hour (IQ 25/75 = 5/18) hours after the TBI. No significant correlation was observed between the admission GCS and the serum TNF-α (Pearson's correlation coefficient = - 0.12, p = 0.18). The serum TNF-α was determined by ELISA.

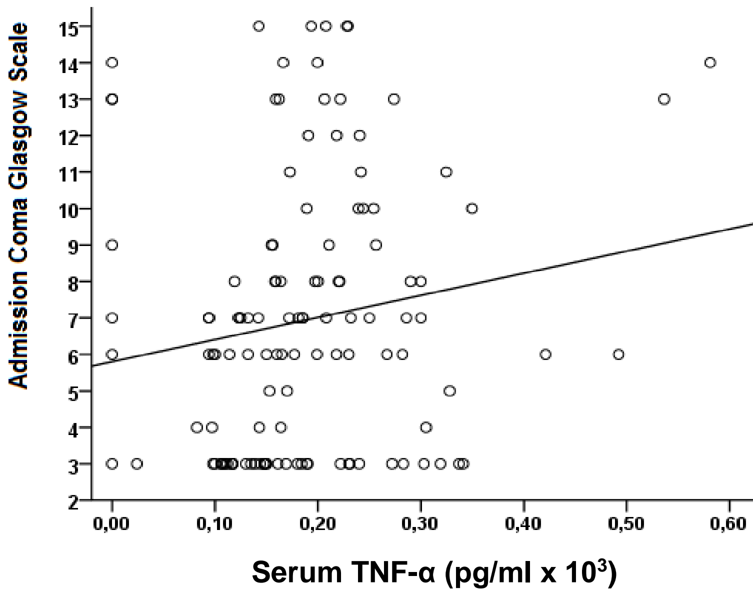


Figure 1D: Serum levels of TNF-α (pg/mL) determined by ELISA measured 11 (IQ 25/75 = 4/13) hours after the TBI in patients with severe (survivors and non-survivors), moderate and mild TBI. There were no statistical difference in the serum levels of TNF-α among patients with severe (survivors and non-survivors), moderate and mild TBI ($p = 0.18$). Data expressed in median and Inter-quartile range. The serum levels of TNF-α were not correlated with the serum level of IL-10 (Sperman's correlation coefficient = - 0.077, $p = 0.23$).

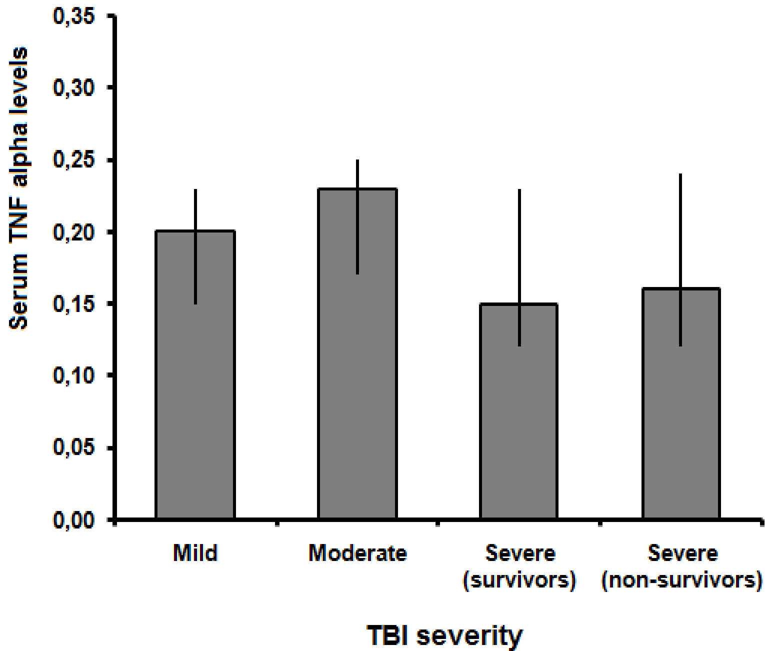


Table 1A: Clinical, demographic, radiological, neurosurgical variables and serum IL-10 levels according to the hospital mortality.

Variable	Outcome			Crude OR For Death (95 CI)	*p* Value
	All Patients *n* = 93 (%)	Survivors *n* = 58 (%)	Non-survivors *n* = 35 (%)		
Age					
Mean (± SD)	35.4 (15.7)	34.0 (15.9)	37.5 (17.0)	N.A.	
18 to 44 years	67 (72.0)	45 (77.6)	22 (62.9)	1.0	
More than 44 years	26 (28.0)	13 (22.4)	13 (37.1)	2.0 (0.8 – 5.1)	0.13
Gender					
Male	77 (82.8)	49 (84.5)	28 (80.0)	1.0	
Female	16 (17.2)	09 (15.5)	07 (20.0)	1.4 (0.5 – 4.0)	0.58
Marshall CT classification [a]					
Type I injury	04 (4.3)	03 (5.2)	01 (2.9)	1.0	
Type II injury	22 (23.7)	16 (27.6)	06 (17.1)	1.1 (0.1 – 13.0)	0.92
Type III injury	14 (15.1)	09 (15.5)	05 (14.3)	1.7 (0.1 – 20.5)	0.69
Type IV injury	07 (7.5)	05 (8.6)	02 (5.7)	1.2 (0.1 – 19.6)	0.90
Evacuated mass lesion	21 (22.6)	13 (22.4)	08 (22.9)	1.8 (0.2 – 20.9)	0.62
Non-evacuated lesion	22 (23.7)	10 (17.2)	12 (34.3)	3.6 (0.3 – 40.2)	0.29
SAH [a]					
No	39 (41.9)	25 (43.9)	14 (41.2)	1.0	
Yes	52 (55.9)	32 (56.1)	20 (58.8)	1.10 (0.6 – 2.7)	0.82
Associated trauma					
Yes	32 (34.4)	21 (36.2)	11 (31.4)	1.0	
No	61 (65.6)	37 (63.8)	24 (68.6)	1.2 (0.5 – 3.0)	0.64
Admission GCS					
7 or 8	28 (30.1)	22 (37.9)	06 (17.1)	1.0	
5 or 6	21 (22.6)	16 (27.6)	05 (14.3)	1.1 (0.3 – 4.4)	0.85
3 or 4	44 (47.3)	20 (34.5)	24 (68.6)	4.4 (1.5 – 13.0)	0.007
Admission Pupils					
Isocoric	45 (48.4)	37 (63.8)	08 (22.9)	1.0	
Abnormal	48 (51.6)	21 (36.2)	27 (77.1)	5.9 (2.3 – 15.4)	< 0.001
Serum cytokines in the 1rst sample	n = 93	n = 58	n = 35		
Hours after TBI (IQ 25-75)	10 (5 - 18)	10 (5 – 22)	10 (4 – 15)	N.A.	0.22

Variable	Outcome: All Patients n = 93 (%)	Survivors n = 58 (%)	Non-survivors n = 35 (%)	Crude OR For Death (95 CI)	p Value
Serum IL-10 levels, pg/ml (IQ 25-75)	0.08 (0.05 - 0.16) [d]	0.07 (0.04 - 0.12)	0.11 (0.06 – 0.24)	N.A.	< 0.0001
IL-10 < 50	21 (22.6)	19 (32.2)	02 (5.7)	1.0	
IL-10 ≥ 50 ≤ 90	33 (35.5)	21 (35.6)	13 (37.1)	6.2 (1.2 – 31.1)	0.03
IL-10 > 90	39 (41.9)	19 (32.2)	20 (57.1)	10.0 (2.0 – 48.9)	0.004
Serum TNF-α, pg/ml (IQ 25-75)	0.16 (0.12 – 0.23)	0.16 (0.12 – 0.23)	0.16 (0.12 – 0.20)	N.A.	0.78
Serum cytokines in the 2nd sample [b]	n = 76	n = 47	n = 29		
Hours after TBI (IQ 25-75)	30 (22 – 37) [e]	30 (21 – 37)	27 (22 – 35)	N.A.	0.37
Serum IL-10 levels, pg/ml (IQ 25-75)	0.07 (0.04 – 0.11)	0.07 (0.04 - 0.09)	0.09 (0.05 – 0.15)	N.A.	0.02
IL-10 < 50	23 (30.3)	16 (34.0)	07 (24.1)	1.0	
IL-10 ≥ 50 ≤ 90	29 (31.2)	20 (42.6)	09 (25.7)	1.0 (0.3 – 0.4)	0.96
IL-10 > 90	24 (25.8)	11 (23.4)	13 (44.8)	2.7 (0.8 – 8.9)	0.10
Serum TNF-α, pg/ml (IQ 25-75)	0.21 (0.18 – 0.25) [f]	0.21 (0.19 – 0.26)	0.21 (0.17 – 0.23)	N.A.	0.55
Serum IL-10 in the 3rd sample [c]	n = 44	n = 31	n = 13		
Hours after TBI (IQ 25-75)	68 (55 – 78)	71 (61 – 80)	67 (54 – 76)	N.A.	
Serum IL-10 levels, pg/ml (IQ 25-75)	0.04 (0.03 – 0.07)	0.04 (0.03 – 0.06)	0.06 (0.03 -0.10)	N.A.	0.28
IL-10 < 50	25 (26.9)	19 (61.3)	06 (46.2)	1.0	
IL-10 ≥ 50 ≤ 90	13 (29.5)	09 (29.0)	04 (30.8)	1.4 (0.3 – 6.2)	0.65
IL-10 > 90	06 (13.6)	03 (09.7)	03 (23.1)	3.2 (0.5 – 20.0)	0.22

[a] Computed tomography was not analyzed in 2 cases; [b] Serum IL-10 were not examined in the second blood sample in 17 patients. Seven patients died before the sampling time, and in eleven the second sample was not collected by the research team; [c] Serum IL-10 was not examined in the third day in 49 patients. Then died before the sampling time and in 39 the second sample was not collected by the research team. [d] Serum IL-10 levels decreased significantly in the second and third sample in comparison to the first sample (≤ 0.005). [e] Serum IL-10 levels in the third sample was lower than in the second blood sample (p < 0.05). [f] There was a significant enhancement in the TNF-α level of second sample in comparison to the first blood sample. TNF-α was not measured in the third blood sample.

Table 1B : Independent association between serum IL-10 levels measured in the first and second days after the severe TBI and hospital mortality.

Variable	All Patients n = 93 (%)	Outcome: Survivors n = 58 (%)	Outcome: Non-survivors n = 35 (%)	Adjusted OR [a] For Death (95% CI)	p Value	Adjusted OR [b] For Death (95% CI)	p Value
Age, years							
18 to 44 years	67 (72.0)	45 (77.6)	22 (62.9)	1.0		1.0	
More than 44	26 (28.0)	13 (22.4)	13 (37.1)	1.9 (0.7 – 5.5)	0.23	2.8 (0.8 – 9.3)	0.09
Associated trauma							
Yes	32 (34.4)	21 (36.2)	11 (31.4)	1.0		1.0	
No	61 (65.6)	37 (63.8)	24 (68.6)	1.2 (0.4 – 3.9)	0.80	1.4 (0.4 – 5.5)	0.60
Admission GCS							
5 or higher	45 (48.10)	35 (60.3)	10 (28.6)	1.0		1.0	
Lower than 5	48 (51.6)	23 (39.7)	25 (71.4)	2.85 (1.1 – 8.0)	0.04	4.6 (1.4 – 15.6)	0.01
Admission Pupils							
Isocoric	45 (48.4)	37 (63.8)	08 (22.9)	1.0		1.0	
Abnormal	48 (51.6)	21 (36.2)	27 (77.1)	4.9 (1,7 – 14.1)	0.003	7.6 (2.1 – 28.0)	0.002
Serum IL-10 in the 1[rst] blood sample	(n = 93)	n = 58	n = 35				
IL-10 < 50	21 (22.6)	19 (32.2)	02 (5.7)				
IL-10 ≥ 50 ≤ 90	33 (35.5)	21 (35.6)	13 (37.1)	4.8 (0.84 – 27.7)	0.08	N.A.	N.A.
IL-10 > 90	39 (41.9)	19 (32.2)	20 (57.1)	6.2 (1.1 – 34.3)	0.03	N.A.	N.A.
Serum IL-10 in the 2nd blood sample	(n = 76)	n = 47	n = 29				
IL-10 < 50	23 (30.3)	16 (34.0)	07 (24.1)	N.A.	N.A.	1.0	
IL-10 ≥ 50 ≤ 90	29 (31.2)	20 (42.6)	09 (25.7)	N.A.	N.A.	2.1 (0.5 – 9.2)	0.33
IL-10 > 90	24 (25.8)	11 (23.4)	13 (44.8)	N.A.	N.A.	5.4 (1.2 – 25.1)	0.03

[a] Adjusted OR for age, associated trauma, admission GCS, admission pupils and serum IL-10 measured in the first collected blood sample. [b] Adjusted OR for age, associated trauma, admission GCS, admission pupils and serum IL-10 measured in the second collected blood sample. Serum IL-10 was not examined in the second day in 17 patients because seven patients died before the sampling time and in eleven the second blood sample was not collected.

REFERENCES

1. Marshall,LF, Becker,DP, Bowers,SA, Cayard,C, Eisenberg,H, Gross,CR, *et al* (1983a) The National Traumatic Coma Data Bank. Part 1: Design, purpose, goals, and results. J. Neurosurgery 59:276-284.

2. Marshall,LF, Toole,BM, Bowers,SA (1983b) The National Traumatic Coma Data Bank. Part 2: Patients who talk and deteriorate: implications for treatment. J. Neurosurgery 59: 285-288.

3. Martins ET, Linhare MN, Sousa DS, Schroeder HK, Meinerz J, Bertotti MM, Gullo J, Hohl A, Dal-Pizzol F, Walz R (2009) Mortality in severe traumatic brain injury: a multivariated analysis of 748 patients from Florianópolis city. Journal of Trauma 67(1):85-90.

4- Schmidt OI, Infanger M, Heyde CE, Ertel W, Stahel PF: The role of neuroinflammation in traumatic brain injury. *Eur J Trauma* 2004, 3:135-149.

5 - LuJ, Goh SJ, Tng PY, Deng YY, Ling EA, Moochhala S: Sistemic inflammatory response following acute traumatic brain injury. *Front Biosci* 2009; 14:3795-3813.

6 - Morganti-Kossmann MC, Satgunaseelan L, Bye N, Kossmann T: Modulation os immune response by head injury. *Injury* 2007, 38:1392-1400.

7 -Stein SC, Smith DH: Coagulopathy in traumatic brain injury. *Neurocrit Care* 2004, 1:479-488.

8- Lehnardt S: Innate immunity and neuroinflamation in the CNS: The role of microglia in Toll-like receptor-mediated neuronal injury. *Glia* 2009, 58:253-263.

9 - Kadhim HJ, Duchateau J, Sebire G: Cytokines and brain injury: invited review. *J Intensive Care Med* 2008, 23: 236-249.

10 - Soriano SG, Piva S. Central nervous system inflammation *Eur J Anaesthesiol Suppl* 2008, 42: 154-159.

11 -Engelhardt B. Immune cell entry into the central nervous system involvement of adhesion molecules and chemokines *J Neurol Sci 2008,* 274: 23-26.

12 - Scholz M, Cinatl J, Schadel-Hopfner M, Windolf J:Neutrophils and the blood-brain barrier dysfunction after trauma. *Med Res Rev* 2007, 27:401-416.

13 - Schmidt OI, Heyde CE, Ertel W, Stahel PF: Closed head injury- an inflammatory disease? *Brain Res Brain Res Rev* 2005, 48:388-399.

14 -D'Mello C, Le T, Swain MG: Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factor alpha signaling during peripheral orgam inflammation. *J Neurosci* 2009, 29:2089-2102.

15 - Kirchhoff C, Buhmann S, Bogner V, Stegmaier J, Leidel BA, Braunstein V, Mutschier W, Bibrthaler P: Cerebrospinal IL10 concentration is elevated in non-survivors as compared to survivors after severe traumatic brain injury. *Eur J Med Res* 2008, 13:464-468.

16- Laird MD, Vender JR, Dhandapani KM: oppsing roles for reactive astrocytes following traumatic brain injury *Neurosignals* 2008, 16:154-164.

17 - Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T: IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF beta1 and blood-brain barrier function. *J Neuroimmunol* 1999, 101:211-221.

18 - Woiciechowsky C, Asadullah K, Nestler D, Eberhardt B, Platzer C, Schoning B, Glockner F, Lanksch WR, Volk HD, Docke WD: Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury. *Nat Med* 1998, 4:808-813.

19. Maas AI, Steyerberg EW, Butcher I, et al. Prognostic value of computerized tomography scan characteristics in traumatic brain injury: results from the IMPACT study. *J Neurotrauma.* 2007;24:303–314.

20. Marmarou A, Lu J, Butcher I, et al. Prognostic value of the Glasgow Coma Scale and pupil reactivity in traumatic brain injury assessed pre-hospital and on enrollment: an IMPACT analysis. *J Neurotrauma.* 2007;24:270–280.

21. McHugh GS, Engel DC, Butcher I, et al. Prognostic value of secondary insults in traumatic brain injury: results from the IMPACT study. *J Neurotrauma.* 2007;24:287–293.

22. Murray GD, Butcher I, McHugh GS, et al. Multivariable prognostic analysis in traumatic brain injury: results from the IMPACT study. *J Neurotrauma.* 2007;24:329 –337.

23. Van Beek JG, Mushkudiani NA, Steyerberg EW, et al. Prognostic value of admission laboratory parameters in traumatic brain injury: results from the IMPACT study. *J Neurotrauma.* 2007;24:315–328.

24. Dziurdzik P, Krawczyk L, Jalowiecki P, Kondera-Anasz Z and Menon LSerum interleukin-10 in ICU patients with severe acute central nervous system injuriesInflammation Research . 2004. Vol 53: 338-343.

25. Bell MJ, Kochanek PM, Doughty LA, et al. Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. *J Neurotrauma*. 1997;14(7):451–457.

26. Neidhardt R, Keel M, Steckholzer U, et al. Relationship of interleukin-10 plasma levels to severity of injury and clinical outcome in injured patients. *J Trauma*. 1997;42(5):863–870[discussion 870-1.

27. Shiozaki T, Hayakata T, Tasaki O, et al. Cerebrospinal fluid concentrations of anti-inflammatory mediators in early-phase severe traumatic brain injury. *Shock*. 2005;23(5):406–410.

28. Knoblach SM, Faden AI. Interleukin-10 improves outcome and alters proinflammatory cytokine expressi expression after experimental traumatic brain injury. *Exp Neurol*. 1998;153(1):143–151.

7.2- ARTIGO 2

Situação: Artigo submetido

Plasma Levels of Lipid and Protein Peroxidation and Mortality in Severe Head Injury: a Multivariate Analysis

Alexandre Hohl,[1,2] Jackson da Silva Gullo,[1] Cláudia Carvalho Pestana Silva,[1] Melina Moré Bertotti,[1,5] Francine Felisberto,[2] Jean Nunes,[1] Bruna de Souza,[2] Fabricia Petronilho,[3] Flávia Mahatma Schneider Soares,[1] Rui Prediger,[1,4] Felipe Dal Pizzol,[3] Marcelo Neves Linhares,[1,5,6] Roger Walz[1,2,6]

1. Centro de Neurociências Aplicadas (CeNAp), Hospital Universitário (HU), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil.
2. Departamento de Clínica Médica, HU, UFSC, Florianópolis, SC, Brazil.
3. Laboratório de Fisiopatologia Experimental, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil;
4. Laboratório de Doenças Neurodegenerativas, Departamento de Farmacologia, UFSC, Florianópolis, SC, Brazil.
5. Serviço de Neurocirurgia, Hospital Governador Celso Ramos (HGCR), Florianópolis, SC, Brazil.
6. Centro de Epilepsia do Estado de Santa Catarina (CEPESC), HGCR, Florianópolis, SC, Brazil.

Corresponding author: Prof. Dr. Roger Walz, M.D., Ph.D., Departamento de Clínica Médica, Hospital Universitário, 3º andar, UFSC, Campus Universitário, Trindade Florianópolis, SC, Brazil.

The first two authors contributed equally to this work.

Introduction: Traumatic brain injury (TBI) is the most common cause of death and incapacity in young people. Experimental studies have demonstrated that oxidative stress is involved in TBI. Yet the association between the damage caused by protein oxidative proteins and lipid-based biomarkers and the prognosis of TBI in humans remains inconclusive in the medical literature.

Objectives: To investigate the association between plasma levels of lipid and protein peroxidation biomarkers with the mortality of patients with severe TBI at the moment of hospital discharge.

Methods: The plasma levels of lipid (TBARS, thiobarbituric acid reactive species) and protein (carbonyl) peroxidation markers of severe TBI patients (GCS ≤ 8) were compared to age- and sex-matched healthy controls. The association between mortality at discharge time of 79 consecutive patients admitted with severe TBI and plasma levels of TBARS and carbonyl measured in the first, second and third day after the TBI were analyzed. Imbalances in the clinical, demographical, radiological, hemodynamic, laboratorial, neurosurgical variables between the survivors and non-survivors were corrected by multiple logistic regression analysis.

Results: The mean age of patients was 34.8 years. Eighty-six percent were male. The mortality was thirty-five percent. The TBARS levels measured in median 12 (IQ 25/75, 6.5 – 19.0), 30 (IQ 25/75, 24.7 – 37.0) and 70 (IQ 25/75, 55.0 – 78.5) hours after TBI were significantly higher than in the control group ($p < 0.01$). There was a progressive enhancement in the plasma levels of TBARS and carbonyl between 12 to 70 hours after TBI. Carbonyl levels of these patients were significantly higher than in controls at 70 ($p = 0.03$), but not at 12 ($p = 0.29$) or 30 ($p = 0.12$) hours after TBI. The univariate analysis showed a trend ($p = 0.09$) for higher plasma levels for both TBARS and carbonyl proteins at 12 hours, but not at 30 or 70 hours after trauma in survivors when compared with non-survivors. These findings were not confirmed after the adjustments by multiple logistic regression analysis. The final model of binary multiple logistic regression showed an adjusted odds

ratio for death 4 times higher in mortality for patients with admission GCS lower than 5 (OR 4.04, CI 95% 1.33 – 12.13, p = 0.01) in comparison to those with higher GCS scores. Abnormal pupils at admission were 4 times more associated with mortality than isocoric pupils (OR = 3.97, CI 95% 1.22 – 12.13, p = 0.02). There was a non-significant trend for association between glucose ≥ 150 mm/dL in the first 12 hours and death than glucose levels between 70 and 149 mg/dL (OR = 2.92, CI 95% 0.96 – 9.02, p = 0.06).

Conclusions: Plasma levels of TBARS and protein carbonyl increase significantly in the first 70 hours after severe TBI but are not independently associated with mortality at the time of discharge. The association between serum levels of oxidative stress markers and both early and long-term morbidity, considering motor abilities, cognitive, psychiatric and quality of life, in patients with TBI, remains to be investigated.

Key words: Traumatic brain injury; prognosis; TBARS; carbonyl; oxidative stress.

INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of morbidity and mortality of young people worldwide [1,2,3,4]. TBI is more frequent among people living in low- and middle-income countries, including Brazil [3,5,6]. After the primary injury related to the trauma itself (contusions, lacerations, hemorrhage), lesions may be aggravated at pre-hospital level or after hospital admission due to hemodynamic imbalance, intracranial hypertension, anemia, infections, fever, hypoxia, seizures, metabolic and electrolytic imbalance.

The primary damage and its subsequent imbalance may activate both secondary injury and neuroprotective cascades, which will interact with a complex biochemical network leading to neuronal and glial survival or death due to necrosis and apoptosis [7,8,9,10].

An excessive amount of reactive oxygen species (ROS), generated by several combined mechanisms, including neutrophils activation, endothelial cells, nerve and glial cells, iron ions (from hemoglobin degradation in the hemorrhagic areas) and brain reperfusion [7,8,10,11] has been implicated in brain lesion in TBI.

The ROS reaction with proteins, deoxyribonucleic acids (DNA) and lipids, leading to the oxidative damage of cells and tissues in TBI, has been discussed for more than twenty years [7,12,13]. Experimental models of TBI in rodents demonstrated that the damage caused by the oxidative stress might vary according to the brain structures, trauma intensity and time after injury [14]. The role of ROS brain damage in human TBI has been studied, however the association between its plasma markers and the prognosis in humans remains controversial [15,16,17,18].

Statistical models, which combine two or more variables of patient’s data to predict clinical outcome - also called prognostic models, have been applied in TBI research [3,6]. The investigation of variables associated with prognosis may help in medical decisions as well as in identifying possible therapeutic targets for the patients. The association between oxidative stress markers and the prognosis of human TBI may be affected by several confounding biases in the context of critically ill patients with TBI. The multiple logistic regression analysis is useful to reduce the possibility of confounding bias leading to spurious associations between the studied variables and prognosis. Here we investigate the association between death and oxidative stress estimated by plasma levels of lipids and proteins

peroxidation markers measured in humans at different times after severe TBI using a multiple logistic regression analysis.

SUBJECTS AND METHODS

We included prospectively 79 consecutive adult patients (age 18 to 80 years) with severe TBI admitted to the intensive care unit of the Hospital Governador Celso Ramos between April 2006 and April 2008 and who met the inclusion criteria. This is a public reference hospital for TBI, covering the metropolitan area of Florianopolis city with a population of approximately one million people. The neurosurgical team, the intensive care unit staff, and the research team were the same along this study. The inclusion criteria were Glasgow coma scale (GCS) score 8 or lower after the intensive care admission. Victims of gunshot injury and patients who evolved to brain death before 24 hours of admission were excluded. Informed consent was obtained from families, and patients whose families withdraw consent were not included in the study. The control group (n = 10) included healthy voluntary people from the hospital and laboratory staff of same age and gender.

Death at the time of discharge was the dependent variable. The clinical, demographic, radiological and neurosurgical independent variables analyzed were age, gender, presence of renal failure (underwent peritoneal dialysis or hemodialysis) during patient care, computed tomography (CT) findings (Marshal CT classification and presence of subarachnoid hemorrhage), presence of associated trauma (face, spinal, thorax, abdomen or limbs), admission GCS, and pupil examination at admission.

CT findings were classified in the six categories according to Marshall *et al.* [19]. Blind CT analysis was done before the patient's discharge by one of the researchers (EMT) and confirmed by another, when necessary. Most of these isolated or combined variables were proven to be associated with TBI outcome in our and other populations [1,2,3,6,19,20].

Oxidative stress parameters were determined in blood plasma on the first, second and third day after TBI. The blood sample was collected as soon as possible on the first day, and, according to the research team schedule possibilities, on the second and third days. The time for blood sampling was calculated from the time of injury onwards. The time span

between TBI and blood sampling was determined by one of our researchers (JSG, MMB or CCP). Five milliliters of peripheral venous blood were collected and centrifuged. The plasma was stored for 24h at -20 ^{o}C and then at -70 ^{o}C until biochemical analysis. As an index of lipid peroxidation, the development of thiobarbituric acid reactive species (TBARS) was measured as previously described [14,21,22,23].

Briefly, the samples were analyzed by absorbance at 535 nm and results were expressed as malondialdehyde-equivalents (nm/mg protein). TBARS plasma levels were expressed in nmol/mg protein x 10^{-5}. The oxidative damage to proteins was assessed by determining carbonyl groups based on the reaction with dinitrophenylhydrazine as previously described [14,21,22,23]. Briefly, proteins were precipitated, dissolved in dinitrophenylhydrazine and absorbance was read at 370 nm. Plasma levels of carbonyl groups were expressed in nmol/mg protein x 10^{-14}. The analysis of oxidative stress parameters was carried out by researchers blinded for all the clinical, demographical, neurosurgical, neuroradiological and hemodynamic variables of patients.

The clinical and hemodynamic parameters, evaluated at the time of the blood sampling, were blood pressure, heart rate, respiratory rate, positive end-expiratory pressure. The laboratorial variables, analyzed on the same sample as for TBARS and carbonyl measurements, were arterial blood gas, sodium, potassium, urea, creatinine, hematocrit, hemoglobin, leukocytes, and platelets.

The variables were measured by three researchers (JAG, AH, CCP) using the same standardized protocol previously approved by the Human Research Ethics Committee of the Universidade Federal de Santa Catarina (UFSC).

Statistical Analysis

The univariate analysis was used to determine the association among the clinical, demographical, radiological, and neurosurgical variables, TBARS and carbonyl groups levels, and the mortality at the time of discharge. Continuous variables were analyzed by the non-parametric test (Mann-Whitney test) because of the relative asymmetry

in the number of survivors and non-survivors. Differences in plasma levels of TBARS and carbonyl groups among the three blood samples collected from the patients after the TBI were analyzed by Wilcoxon signed ranks test. Categorical variables were analyzed by binary logistic regression. The magnitude of the association between death and the independent categorical variables was measured by the odds ratio (OR) and respective 95% confidence interval (CI) estimated by unconditional logistic regression.

To identify variables that were independently associated with death, a multiple logistic regression was performed using the following conditional method. For this analysis, continuous variables including the biochemical markers of oxidative stress were categorized. Variables showing an association with death through the univariate analysis with a "p" level of significance lower than 0.20 were included in the analysis. The variable "associated trauma" was also included in the multivariate analysis. The probability of stepwise entry was 0.05 and removal 0.10. The classification cutoff was 0.5 with maximum interactions of 20. "P" levels lower than 0.05 were considered significant. To avoid type II error, we did not adjust for multiple tests using a more stringent criterion for the "p" level [24]. Missing cases were clearly described in the results and tables. Statistical analysis was done using the SPSS program 10.0 (Chicago, IL).

RESULTS

The clinical, demographic, radiological, neurosurgical variables and the mortality of patients at the time of discharge are shown in Table 1. The mean age was 34.8 years for patients and 29.6 for controls ($p = 0.30$). Eighty-six percent of patients and eighty percent of controls were male ($p = 0.45$). Twenty-eight patients died (35.4%). The mean hospitalization time was 20 (± 18) days, 29 (± 21) days for survivors and 6 (± 5) days for non-survivors.

The causes of death were TBI itself for 25 patients (89.3%). One patient died due to heart infarction (3.6%), one due to pneumonia, pleural effusion and cardiac arrest (3.6%) and one due to pneumonia evolving to sepsis, renal failure and cardiac arrest (3.6%). These 3 patients died after the third blood sample collection. There was no association between death and age, gender, presence of renal failure, admission, tomographic findings according to Marshal classification and sub-arachnoid hemorrhage, and associated trauma ($p>0.23$). There was a significant association between death and lower scores in the Glasgow

Coma Scale (p=0.006), anisocoric (p=0.04) and midriatic pupils (p=0.001) at admission. These analyses remain unaltered when excluding deaths not attributed to TBI (data not shown).

Plasma levels of TBARS or carbonyl groups of the TBI patients and control group, according to the prognosis (death at time of discharge), are shown in Table 2. TBARS levels measured 12, 30 and 70 hours after TBI were significantly higher than in the controls ($p < 0.01$). Carbonyl levels measured 12 hours after TBI did not rise significantly in comparison to the controls ($p = 0.29$). However, there was a trend for higher levels of carbonyl groups measured 30 hours after TBI in comparison to the control group ($p = 0.12$). Carbonyl levels of patients at 70 hours after TBI were significantly higher than those of controls ($p = 0.03$). There was a trend ($p=0.09$) for higher plasma levels for both TBARS and carbonyl proteins at 12 hours, but not at 30 or 70 hours ($p>0.25$) after trauma in survivors when compared to non-survivors. A subset analysis of patients with isolated TBI showed no significant association between the studied biomarkers and death ($p > 0.20$).

In patients, TBARS plasma levels did not change significantly ($p = 0.23$) between the first and the second blood sampling. However, TBARS plasma levels increased significantly 70 hours after TBI ($p<0.001$) in comparison to 12 and 30 hours after severe TBI. There was a trend ($p=0.08$) for higher plasma levels of carbonyl groups measured at 30 hours when compared to levels at 12 hours after severe TBI. At 70 hours after TBI, plasma levels of carbonyl groups rose significantly in comparison to 30 ($p=0.02$) and 12 ($p<0.0001$) hours after the severe TBI. There were no statistical differences between the time of the first ($p>0.69$) and second ($p=0.71$) blood sampling after TBI in survivors and non-survivors. In comparison to survivors, there was a trend for shorter time between TBI and the third blood sampling in non-survivors ($p = 0.06$).

One patient died before the second and nine patients passed away before the third sampling. Due to some difficulties in the schedule of the researchers' team, the second blood sample was not collected in nine patients and the third blood sample was not collected in twelve patients. These blood-sampling failures occurred by chance and were not related to any clinical or laboratorial criteria that could affect the patient's prognosis.

There were no statistical differences in plasma levels of TBARS and protein carbonyl groups at the second and third sampling between survivors and non-survivors. The association between prognosis and plasma markers of oxidative stress damage, measured on the third

sample collected, remained non significant after correcting the imbalance in blood sampling time after TBI (p = 0.63, data not shown).

Table 3 shows the association between death, clinical and laboratorial parameters at the time of the first blood sampling. This analysis was carried out to identify imbalances between survivors and non-survivors of variables that could affect plasma levels of the oxidative stress markers leading to a spurious association between oxidative stress and prognosis.

The laboratorial variables were measured on the same blood sample used to determine plasma TBARS and carbonyl levels 12 hours after TBI. Patients who died had significantly higher levels of pH (p=0.01), glucose (p= 0.04) and sodium than survivors. Non–survivors had significantly lower levels of hematocrit and hemoglobin than those who survived.

Table 4 shows the final model of multiple logistic regression analysis that better explains the independent association among each categorical variable and death at the time of discharge. This analysis showed that plasma levels of TBARS and carbonyl groups were not independently associated with death (they were not included in the final model). Higher levels of serum glucose 12 hours after TBI (p =0.04), lower GCS (p =0.04) and abnormal pupils (p = 0.006) at admission were independently associated with death.

DISCUSSION

Our results demonstrated that plasma levels of TBARS and carbonyl groups are significantly higher in the TBI group than in the healthy controls group suggesting an association between oxidative stress damage of lipids and proteins and severe TBI in humans. Rise in TBARS levels occurred earlier (during the first 12 hours) than in carbonyl levels (second and third day after severe TBI). In an experimental model, we demonstrated that both TBARS and carbonyl levels increased in a time-dependent manner in different brain regions of rodents submitted to experimental TBI [14].

Our findings are in agreement with previous studies showing an enhancement of oxidative stress damage in patients with severe TBI [15]. In fact, the brain is particularly vulnerable to oxidative stress because of its higher rate of oxygen consumption, higher level of transition metals, and polyunsaturated fatty acids. The oxidative stress pathways may be activated by excitatory

mechanisms as well as several secondary mechanisms including reperfusion, and iron in its reduced state resulting from hemoglobin degradation (Fenton reaction) [14]. The oxidative damage contributes to mitochondrial dysfunction leading to energy failure. This could trigger a complex cascade of cellular events, such as cellular depolarization and Ca^{+2} influx leading to excitotoxic and apoptotic neuronal death [14,25].

Using the same biochemical technique as for plasma TBARS determination, Scholpp *et al.* demonstrated that there was no difference in the levels of this plasma marker for lipid peroxidation between patients with TBI 12 to 24 hours after admission and healthy controls, thus suggesting it is not a useful biomarker for oxidative stress damage in TBI [18]. However, in this study the patient sample was relatively small (n = 18) showing a variable degree in TBI severity (GCS between 3 and 13).

The trend shown by our data for association between plasma levels of TBARS and carbonyl groups at 12 hours and mortality in severe TBI did not become significant after the multiple logistic regression analysis. This is in contradiction to the previous findings of Nayak *et al.* [15,16], which demonstrated an association between mortality and higher levels of TBARS from the erythrocytes of severe TBI patients.

The increase in plasma TBARS and carbonyl levels may also be related to oxidative stress damage occurring in other organs such as lung, liver, muscles, gastrointestinal tract of patients with severe TBI associated with multiple trauma, infections, and hypoxia. Confounding bias may influence the interpretation of association studies between oxidative stress markers and the prognosis of TBI. Several clinical, laboratorial, neurosurgical and radiological variables are independently associated with the mortality of severe TBI patients at time of discharge [3]. We believe the imbalance in the distribution of admission GCS or pupillary abnormalities in

the small sample of patients of Nayak *et al.* (6 deaths, 24 with severe sequelae and 24 with good recovery) could explain their findings [15,16]. The use of multiple logistic regression analysis in a large sample of patients may help determine the independent association of erythrocyte TBARS levels and prognosis in human TBI. Differences in the sensitivity of the applied techniques for TBARS measurements (plasma and erythrocitary) also need to be considered and studies determining the sensibility and specificity of both techniques may help to clarify this point. Besides, ten patients died and therefore did not have their blood drawn. This potential bias cannot be adequately controlled. If there were an association between the biomarkers and mortality, this differential loss could have contributed to the lack of significant findings. The wide range of TBARS and carbonyl levels in the patient group may lead to a reduced power of the study, and may also be a limitation for the use of these biomarkers.

Our findings indicate that, although patients with TBI had a progressive increase in the level of plasma markers for oxidative stress damage at the first three days after the injury, this is probably not a determinant of mortality. Some recent studies have demonstrated that excitatory amino acids, which are involved in the core mechanisms of neuronal injury due to excitotoxicity, also trigger neuroprotective signals against oxidative stress [26]. If neuroprotective pathways are activated proportionally to the level of injury, which in turn is associated with plasma TBARS and carbonyl levels, than the mortality of patients with higher levels of those biomarkers may be the same as of patients with lower levels. We replicated ours [3] and other previous studies [27] showing that higher levels of serum glucose 12 hours after the TBI, lower GCS and abnormal pupils at admission [3,27,28] remain independently associated with death. In the present study, there was no association between CT, SAH and death as previously demonstrated in our population. This is probably due to the small sample size of the present study. The same hypothesis may explain the trend for higher mortality in patients admitted with anisocoric pupils as compared to those having isocoric ones. Injury severity score (or any other score such as TRISS or RTS) has been demonstrated in other studies to be correlated with mortality and may be a better outcome predictor than

associated trauma alone. This variable will be included in further studies.

In conclusion, the increase in plasma TBARS and protein carbonyl levels seen in the first 70 hours after severe TBI is not independently associated with mortality at the time of discharge. The association between serum levels of the oxidative stress markers and both early and long-term prognosis including fine motor abilities, cognitive, psychiatric and quality of life aspects [4] remains to be investigated in patients with TBI.

ACKNOWLEDGMENTS: This work was supported by CNPq (Brazilian Council for Scientific and Technologic Development, Brazil), FAPESC (Foundation for Scientific Research and Technology of the State of Santa Catarina). Our thanks to Saulo Correa de Melo, Laboratório Santa Luzia.

REFERENCES

1- Marshall LF, Becker DP, Bowers SA, Cayard C, Eisenberg H, Gross CR, Grossman, RG, Jane, JA, Kunitz, SC, Rimel, R, Tabaddor, K, Warren, J. The National Traumatic Coma Data Bank. Part 1: Design, purpose, goals, and results J Neurosurg. 1983; 276-284.

2- Marshall, LF, Toole, BM, Bowers, SA. The National Traumatic Coma Data Bank. Part 2: Patients who talk and deteriorate: implications for treatment J Neurosurg. 1983; 285-288.

3- Martins, ET, Linhares, MN, Sousa, DS, Schroeder, HK, Meinerz, J, Rigo, LA, Bertotti, MM, Gullo, J, Hohl, A, Dal-Pizzol, F, Walz, R. Mortality in severe traumatic brain injury: a multivariated analysis of 748 Brazilian patients from Florianopolis City J Trauma. 2009; 85-90.

4- Schwarzbold M, Diaz A, Martins ET, Rufino A, Amante LN, Quevedo J, Hohl A, Linhares MN, Walz R. Psychiatric disorders and traumatic brain injury. Neuropsychiatr Dis Treat. 2008; 1176-6328.

5- Koizumi MS, Lebrao, ML, Mello-Jorge, MH, Primerano, V. Morbidity and mortality due to traumatic brain injury in Sao Paulo City, Brazil, 1997 Arq. Neuropsiquiatr. 2000; 81-89.

6- Perel, P, Edwards, P, Wentz, R, Roberts, I. Systematic review of prognostic models in traumatic brain injury BMC Med Inform Decis Mak. 2006; 38.

7- Friedlander, RM. Apoptosis and caspases in neurodegenerative diseases N Engl J Med. 2003; 1365-1375.

8- Graham, DP, Cardon, AL. An update on substance use and treatment following traumatic brain injury Ann N Y Acad Sci. 2008; 148-162.

9- LaPlaca, MC, Simon, CM, Prado, GR, Cullen, DK. CNS injury biomechanics and experimental models Prog Brain Res. 2007; 13-26.

10- Spaethling, JM, Geddes-Klein, DM, Miller, WJ, von Reyn, CR, Singh, P, Mesfin, M, Bernstein, SJ, Meaney, D. F. Linking impact to cellular and molecular sequelae of CNS injury: modeling in vivo complexity with in vitro simplicity Prog Brain Res. 2007; 27-39.

11- Ikonomidou, C, Turski, L. Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? Lancet Neurol. 2002; 383-386.

12- Braughler, JM, Hall, ED. Central nervous system trauma and stroke. I. Biochemical considerations for oxygen radical formation and lipid peroxidation Free Radic Biol Med. 1989; 289-301.

13- Hall, ED, Braughler, JM. Central nervous system trauma and stroke. II. Physiological and pharmacological evidence for involvement of oxygen radicals and lipid peroxidation Free Radic Biol Med. 1989; 303-313.

14- Petronilho F, Feier G, de Souza B, Guglielmi C,Constantino LS, Walz R,Quevedo J,Dal-Pizzol Oxidative Stress in Brain According to Traumatic Brain Injury Intensity. J Surg Res. 2009.

15- Nayak C, Nayak D, Bhat S, Raja A, Rao A. Relationship between neurological outcome and early oxidative changes in erythrocytes in head injury patients Clin Chem Lab Med. 2007; 629-633.

16- Nayak CD, Nayak DM, Raja A, Rao A. Time-relative changes in the erythrocyte antioxidant enzyme activities and their relationship with Glasgow Coma Scale scores in severe head injury patients in the 21-day posttraumatic study period. Indian J Med Sci. 2007; 381-389.

17- Nayak CD, Nayak DM, Raja A, Rao A. Erythrocyte indicators of oxidative changes in patients with graded traumatic head injury. Neurol India. 2008; 31-35.

18- Scholpp J, Schubert JK, Miekisch W, Noeldge-Schomburg GF. Lipid peroxidation early after brain injury. J Neurotrauma. 2004; 667-77.

19- Marshall LF, Marshall SB, Klauber MR, Van Berkum Clark M, Eisenberg H, Jane JA, Luerssen TG, Marmarou A, Foulkes MA. The diagnosis of head injury requires a classification based on computed axial tomography J Neurotrauma. 1992; 287-292.

20- Eisenberg HM, Gary HEJr, Aldrich EF, Saydjari C, Turner B, Foulkes MA, Jane JA, Marmarou A, Marshall LF, Young HF. Initial CT findings in 753 patients with severe head injury. A report from the NIH Traumatic Coma Data Bank J Neurosurg. 1990; 688-698.

21- Dal-Pizzol F, Klamt F, Vianna MM, Schroder N, Quevedo J, Benfato MS, Moreira JC, Walz R. Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpine or kainic acid in Wistar rats Neurosci Lett. 2000;c179-182.

22- Dal-Pizzol F, Quevedo J, Streck E, Walz R, Moreira JC. Changes in lipid composition in hippocampus early and late after status epilepticus induced by kainic acid in wistar rats. Metab Brain Dis. 2007; 25-29.

23- Klamt F, Dal-Pizzol F, Conte da Frota ML, Walz R, Andrades ME, da Silva EG, Brentani RR, Izquierdo I, Fonseca Moreira JC. Imbalance of antioxidant defense in mice lacking cellular prion protein. Free Radic Biol Med. 2001;1137-1144.

24- Perneger TV. What's wrong with Bonferroni adjustments Bmj.1998; 1236-1238.

25- Raghupathi, R. Cell death mechanisms following traumatic brain injury Brain Pathol. 2004; 215-222.

26- Papadia S, Soriano FX, Leveille F, Martel MA, Dakin KA, Hansen HH, Kaindl A, Sifringer M, Fowler J, Stefovska V, McKenzie G, Craigon M, Corriveau R, Ghazal P, Horsburgh K, Yankner BA, Wyllie DJ, Ikonomidou C, Hardingham GE. Synaptic NMDA

receptor activity boosts intrinsic antioxidant defenses Nat Neurosci. 2008; 476-487.

27- Murray GD, Butcher I, McHugh GS, Lu J, Mushkudiani NA, Maas AI, Marmarou A, Steyerberg EW. Multivariable prognostic analysis in traumatic brain injury: results from the IMPACT study. J Neurotrauma 2007; 24(2):329-337.

28- Lieberman JD, Pasquale MD, Garcia R, Cipolle MD, Mark Li P, Wasser TE. Use of admission Glasgow Coma Score, pupil size, and pupil reactivity to determine outcome for trauma patients. J Trauma. 2003; 55(3):437-442; discussion 442-443.

Table 1: Clinical, demographic, radiological, neurosurgical variables and mortality of patients with severe head injury, at the time of discharge.

Variable	All Patients $n = 79$ (%)	Outcome: Survivors $n = 51$ (%)	Outcome: Non-survivors $n = 28$ (%)	Crude OR For Death (95 CI)	p Value
Age					
Mean ± SD	34.8 (15.6)	33.5 (14.2)	37.3 (17.9)	NA	0.43
Gender					
Male	68 (86.1)	45 (88.2)	23(88.1)	1.0	
Female	11 (13.9)	6 (11.8)	5 (17.9)	1.63 (0.45 – 5.92)	0.46
Renal Failure					
Yes	9 (11.4)	6 (11.8)	3 (10.7)	1.0	
No	70 (88.6)	45 (88.2)	25 (89.3)	1.11(0.26 – 4.83)	0.89
Marshall CT classification					
Type I injury	4 (5.1)	3 (5.9)	1 (3.6)	1.0	
Type II injury	21 (26.6)	17 (33.3)	4 (14.3)	0.71 (0.60 – 8.70)	0.79
Type III injury	9 (11.4)	5 (9.8)	4 (14.3)	2.40 (0.17 – 32.90)	0.51
Type IV injury	8 (10.1)	6 (11.8)	2 (7.1)	1.0 (0.06 – 16.0)	1.0
Evacuated mass lesion	15 (19)	10 (19.6)	5 (17.9)	1.5 (0.12 – 18.4)	0.75
Non-evacuated lesion	22 (27.8)	10 (19.6)	12(42.9)	3.6 (0.32 – 40.20)	0.30

Variable	All Patients n = 79 (%)	Outcome		Crude OR For Death (95 CI)	p Value
		Survivors n = 51 (%)	Non-survivors n = 28 (%)		
SAH					
No	35 (44.3)	24 (47.1)	17 (60.7)	1.0	
Yes	44 (55.7)	27 (52.9)	11 (39.3)	1.37 (0.54- 3.50)	0.51
Associated trauma					
Yes	47 (59.5)	33 (64.7)	14 (50.0)	1.0	
No	32 (40.5)	18 (35.3)	14 (50)	1.83 (0.72 – 4.70)	0.23
Admission GCS					
7 or 8	30 (38.0)	24 (47.1)	6 (21.4)	1.0	
5 or 6	16 (20.3)	12 (23.5)	4 (14.3)	1.33 (0.32 – 5.64)	0.69
3 or 4	33 (41.8)	15 (29.4)	18 (64.3)	4.8 (1.55 – 14.81)	0.006
Admission Pupils					
Normal	38 (48.1)	31 (60.8)	07 (25.0)	1.0	
Abnormal	41 (51.9)	20 (39.2)	21 (75.0)	4.65 (1.67 – 12.94)	0.003

Table 2: Plasma levels of TBARS (nmol/mg protein x 10^{-5}) and protein carbonyl (nmol/mg protein x 10^{-14}) of TBI patients and control group.

Biochemical Variables	Control group	All Patients	Outcome		*"p"* level [h]
			Survivors	Non-survivors	
Plasma sample 1	**n = 10**	**n = 79**	**n = 51**	**n = 28**	
Hours after Trauma (Median, IQ 25 – 75)	N.A.	12.08 (6.5 – 19.00)	11.0 (6.5 – 19)	13.67 (6.25 – 17.4)	0.69
TBARS Level (Median, IQ 25 – 75)	14.0 (7.5 – 18.0)	45.86 (14.66 - 237.43) [a]	41.64 (10.19 – 197.10) [b]	57.75 (25.0– 389.54)	0.09
Carbonyl Level (Median, IQ 25 – 75)	20.0 (15.75 – 24.25)	27.53 (10.56 – 161.89)	25.31 (9.26 – 93.04) [d]	54.54 (17.68 – 211.65)	0.09
Plasma sample 2		n = 69 [f]	n = 42	n = 27	
Hours after Trauma (Median, IQ 25 – 75)	N.A.	30.58 (24.75 -37.0)	30.7 (24.0 – 37.0)	29.25 (25.25 – 36.6)	0.71
TBARS Level (Median, IQ 25 – 75)	14.0 (7.5 – 18.0)	38.70 (18.8 – 226.47) [b]	35.2 (16.6- 235.62)	42.4 (20.5 – 223.3)	0.36
Carbonyl Level (Median, IQ 25 – 75)	20.0 (15.75 – 24.25)	44.97 (12.37 – 133.59) [c]	40.05 (12.60 – 108.49)	67.45 (11.22 – 149.89)	0.77
Plasma sample 3		n = 58 [g]	n = 40	n = 18	
Hours after Trauma (Median, IQ 25 – 75)	N.A.	70.0 (55.0 – 78.5)	71.5 (59.4-80.0)	62.5 (51.1 – 71.3)	0.06
TBARS Level (Median, IQ 25 – 75)	14.0 (7.50 – 18.0)	80.49 (58.55 – 382.59) [d]	73.70 (56.2 – 359.6)	92.12 (68.68 – 491.44)	0.37
Carbonyl Level (Median, IQ 25 – 75)	20.0 (15.75 – 24.25)	38.27 (15.93 – 197.17) [e]	38.20 (15.83 – 140.52)	52.77 (26.12 – 295.50)	0.26

[a] Significant increase in plasma TBARS level measured 12 hours after TBI in comparison to the control group (p = 0.003).
[b] Significant increase in plasma TBARS level measured 30 hours after TBI in comparison to the control group (p < 0.0001).
[c] There was a trend for higher levels of carbonyl measured 30 hours after TBI in comparison to the control group (p = 0.12).
[d] Significant increase in plasma TBARS level measured 70 hours after TBI in comparison to the control group (p < 0.0001).
[e] Significant increase in plasma carbonyl level measured 70 hours after TBI in comparison to the control group (p < 0.0001).
[f] The second blood sample was not examined in 10 patients. One patient died before sampling time, and nine had no second sample collected.
[g] The third blood sample was not examined in 21 patients. Nine patients died before the collection time and twelve had no third sample collected.
[h] Comparison between plasma levels of TBARS and carbonyl of survivors and non-survivors analyzed 12, 30 and 70 hours after severe TBI.
TBARS level did not change significantly between 12 and 30 hours after TBI (p = 0.23), but increased significantly at 70 hours in comparison to 12 and 30 hours after TBI (p<0.001).
There was a trend (p=0.08) for higher plasma levels of carbonyl measured at 30 hours in comparison to levels at 12 hours after TBI. TBARS levels increased significantly at 70 hours in comparison to 30 (p=0.02) and 12 (p<0.0001) hours after TBI.

Table 3: Clinical and laboratorial parameters at the time of the first blood sampling

Variable [a]	All Patients	Outcome		*p* Value [f]
	$n = 79$	Survivors $n = 51$	Non-survivors $n = 28$	
Blood Pressure				
Systolic	126.2 (30.4)	128.5 (30.6)	121.9 (30.1)	0.37
Diastolic	73.5 (19.6)	75.8 (19.4)	69.5 (19.7)	0.17
Heart rate	89.6 (22.1)	89.6 (20)	89.5 (26.0)	1.0
Respiratory rate	20.1 (11.0)	20.7 (13.3)	19.0 (4.0)	0.55
PEEP	5.8 (4.4)	6 (5.2)	5.6 (2.4)	0.69
Blood gases [b]				
pH	7.37 (0.08)	7.39 (0.07)	7.33 (0.10)	0.003
pCO_2	32.7 (10.5)	31.5 (6.7)	34.9 (15.1)	0.19
pO_2	260.6 (254.0)	246.1 (121.3)	286.9 (397.3)	0.51
HCO_3^-	18.8 (3.5)	19.2 (3.0)	18.3 (4.2)	0.28
O_2 Sat	97.0 (12.0)	98.2 (4.9)	94.9 (19.1)	0.26
pH < 7.35	25 (31.3%)	11 (22.4)	14 (51.9)	
pH ≥ 7.35	51 (63.8%)	38 (77.6)	13 (48.1)	0.01 [g]

Variable [a]	Outcome			
	All Patients $n = 79$	Survivors $n = 51$	Non-survivors $n = 28$	p Value [f]
Serum Glucose [c]	167.2 (74.3)	153.8 (60.7)	191.8 (90.7)	0.04
Between 70 and 149 mg/dL	38 (48.1%)	30 (62.5%)	8 (30.8%)	
≥150 mg/dL	36 (45.6%)	18 (37.5%)	18 (69.2%)	0.01 [g]
Sodium [d]	141.1(7.1)	139.9 (5.6)	143.4 (9.1)	
< 143	64 (80.0%)	45 (90.0%)	19 (70.4%)	
≥144	13 (16.3%)	5 (10.0%)	8 (29.6%)	0.04 [g]
Potassium	4.4 (4.1)	4.7 (5.1)	3.9 (0.7)	0.48
Urea	29.9 (11.4)	29.6 (10.9)	30.5 (12.8)	0.78
Creatinine	0.93 (0.27)	0.93 (0.25)	0.92 (0.30)	0.74
Hematocrit	32.82 (6.4)	33.75 (6.5)	31.13 (5.88)	0.03
< 31	28 (35.4%)	13 (25.5%)	15 (53.6%)	
≥ 31	51 (64.6%)	38 (74.5%)	13(46.4%)	0.02 [g]
Hemoglobin [e]	11.74 (4.18)	12.37 (4.86)	10.55 (2.10)	0.01
Hb < 11	32 (40%)	16 (32.0%)	16 (59.3%)	
Hb ≥ 11	45 (56.3%)	34 (68.0%)	11(40.7%)	0.03 [g]

Variable [a]	All Patients $n = 79$	Survivors $n = 51$	Non-survivors $n = 28$	p Value [f]
		Outcome		
Total Leukocytes	15846 (6.990)	15475 (7.374)	16490 (6359)	0.56
Platelets	189151 (6.0617)	192370 (5.4513)	183667 (7587)	0.57

[a] All variables are expressed as mean (SD), and some of them are also categorized.
[b] Gasometrical parameters from three patients were not determined.
[c] Serum glucose level from five patients was not determined.
[d] Sodium level from three patients was not determined.
[e] Hemoglobin level from two patients was not determined.
[f] Statistical analysis with Student “t” test.
[g] Statistical analysis with Fisher Exact test.
PEEP = Positive End Expiratory Pressure.

Table 4: Multiple logistic regression model that better explains the association between each categorical variable and death at the time of discharge.

Variable	All Patients *n* = 79 (%)	Outcome: Survivors *n* = 51 (%)	Outcome: Non-survivors *n* = 28 (%)	Crude OR For Death (95% CI)	*P* Level	Adjusted OR[a] for Death (95% IC)	P Level
Serum glucose in the first sample							
70 – 149 mg/dL	38 (48.1)	30 (62.5)	8 (30.8)	1.0		1.0	
≥150 mg/dL	36 (45.6)	18 (37.5)	18 (69.2)	3.75 (1.36 – 10.37)	0.01	2.92 (0.96 – 9.02)	0.06
Admission neurological parameters:							
GCS ≥ 5	46 (48.2)	36 (78.3)	15 (45.5)	1.0		1.0	
GCS < 5	33 (41.8)	10 (21.7)	18 (54.5)	4.32 (1.62 – 11.51)	0.003	4.03 (1.33 – 12.13)	0.01
Admission Pupils							
Normal	38 (48.1)	31 (60.8)	07 (25.0)	1.0		1.0	
Abnormal	41 (51.9)	20 (39.2)	21 (75.0)	4.65 (1.67 – 12.94)	0.003	3.97 (1.22 – 12.13)	0.02

[a] Adjusted OR indicates the magnitude of association between each categorical variable and death, independent of imbalances in the distribution of all other clinical, demographic, radiological, and neurosurgical variables studied.

7.3 - ARTIGO 3

Situação: Publicado na Neurocriticalcare – Fator de impacto 2.17

Neurocrit Care
DOI 10.1007/s12028-010-9462-y

ORIGINAL ARTICLE

Hospital Mortality of Patients with Severe Traumatic Brain Injury is Associated with Serum PTX3 Levels

Jackson da Silva Gullo · Melina Moré Bertotti · Cláudia Carvalho Pestana Silva · Marcelo Schwarzbold · Alexandre Paim Diaz · Flávia Mahatma Schneider Soares · Fernando Cini Freitas · Jean Nunes · José Tadeu Pinheiro · Edelton Flavio Morato · Rui Daniel Prediger · Marcelo Neves Linhares · Roger Walz

Published online: 23 October 2010

7.4- ARTIGO 4

Situação: Publicado na Brain Research - Fator de Impacto: ***2.494*** (JCR-2008)

BRAIN RESEARCH 1302 (2009) 248–255

available at www.sciencedirect.com

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Exercise effects on activities of Na^+,K^+-ATPase, acetylcholinesterase and adenine nucleotides hydrolysis in ovariectomized rats

Juliana Ben[a], *Flávia Mahatma Schneider Soares*[a], *Fernanda Cechetti*[a], *Fernanda Cenci Vuaden*[a], *Carla Denise Bonan*[b], *Carlos Alexandre Netto*[a], *Angela Terezinha de Souza Wyse*[a,*]

[a]*Laboratório de Neuroproteção e Doença Metabólica, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600-Anexo, CEP 90035-003, Porto Alegre, RS, Brazil*

[b]*Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil*

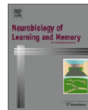
7.5 - ARTIGO 5

Situação: Publicado na Neurobiology of Learning and Memory - Fator de Impacto: ***3.757*** (JCR-2008)

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme



Running exercise effects on spatial and avoidance tasks in ovariectomized rats

Juliana Ben, Flávia M.S. Soares, Emilene B.S. Scherer, Fernanda Cechetti, Carlos Alexandre Netto, Angela T.S. Wyse *

Laboratório de Neuroproteção e Doença Metabólica, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, RS, Brazil

7.6 - ARTIGO 6

Situação: Publicado no International Journal of Developmental Neuroscience – Fator de Impacto: ***1.869*** (JCR-2008)

Hyperhomocysteinemia reduces glutamate uptake in parietal cortex of rats

Cristiane Matté, Ben Hur M. Mussulini, Tiago M. dos Santos, Flávia M.S. Soares, Fabrício Simão, Aline Matté, Diogo L. de Oliveira, Christianne G. Salbego, Susana T. Wofchuk, Angela T.S. Wyse *

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil