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RESEARCH ARTICLE

SERUM BIOMARKER ANALYSIS ON CLINICAL THERAPY STUDY IN SEVERE TRAUMATIC BRAIN INJURY PROGESTERONE EFFECT IN MODULATING S-100B, AQP4 AND IL-6

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ABSTRACT

Background: Traumatic Brain Injury (TBI) consists of two processes primary injury and secondary injury. Secondary injury in TBI involves many factor of molecular and cellular responses to the primary impact. All of these factors culminate in cellular dysfunction and cell death via necrotic and more apoptotic. These downstream molecular and cellular processes in the secondary injury are the focus of many pre-clinical and clinical therapeutic studies. The spatiotemporal distribution of S-100B, IL-6, AQP4 productions in secondary injury are some key marker of acute feature after TBI pathophysiology. By examining changes of this protein in these processes, we can expect to identify novel approaches to TBI intervention. Hypotheticly, posttraumatic suppression of the hypothalamic-pituitary-adrenal axis and an increase in cytokine-mediated peripheral aromatase activity, leading to an imbalance in sex steroid estrogen, progesterone, and testosterone serum levels, have been interest for precise understanding of that mechanisms involved. We treatment analyzed serum biomarkers (S100-B, IL-6, AQP4) as part of a randomize placebo-controlled of progesterone in patients with severe TBI (sTBI), and analyzed the long-term predictive value of these biomarkers on the dichotomized Glasgow Outcome Scale (GOS) score at 3 months. **Method:** This study was part of a prospective, outcome-assessor–and statistician-blinded, randomized, placebo- controlled trial of progesterone. The population age 15 – 60 years patients with severe TBI (sTBI), (Glasgow Coma Scale (GCS) score 4–8, who presented at our care trauma center, Central Hospital Dr Sutomo Surabaya within 24 hours after injury. We obtained approval from institutional ethics committee prior to the trial. Of 40 patients with sTBI, we serially analyzed 3 serum biomarkers S-100B, AQP4, and IL-6. We analyzed the long-term predictive value of serum biomarkers on dichotomized GOS score of 3 months. The serum levels of S-100B, IL-6, AQP4 were determined using a sandwich ELISA technique. The samples for the determination of these biomarkers were taken at the day I (24 hours), immediately after randomization and before Progesterone given intramuscular 1 mg/kgBW single dose, and then day IV (96 hours) later. Using IBM SPSS Statistic software version 22, analysis for 24 hours, 96 hours, and average serum biomarkers stratified according to outcome (The dichotomized GOS) was performed. **Results:** The serum levels S-100B, AQP4 and IL-6 were across the dichotomized GOS groups at 3 months in both groups. GOS 3 months maked two category: Poor outcome (label 1) for GOS score 1 – 3 and good outcome (label 2) for GOS 4 – 5. Binary logistic regression result showed all value biomarker significant model to prediction the GOS dichotomy. Analysis to prediction from good outcome to poor outcome (to right axis direction) we have the simulation equation. Δunfav. = ΔX good - ΔX poor. In the control group: S100B was increase, AQP4 was decrease and IL-6 no change. To analysis effect of progesterone as intervention group we found S-100B was extremely high increased that means progesterone indirectly can reduce neuronal injury. AQP4 and IL-S was decrease compare to control, that mean possibly progesterone have effect modulating up regulation in AQP 4 to inhibit neuroinflamation. **Conclusion:** Serial monitoring of S-100B, IL-6 and AQP4 serum levels could aid in prognostication in patients with sTBI. S-100B is the best good accuracy for predict outcome. Progesterone have an effect in change of S-100B serum level expression that involve in neuronal injury, and the process of cerebral edema (modulating AQP4 link to IL-6). However, in this study Progesterone had no benefit in overall clinical outcome (GOS dichotomy 3 months).

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INTRODUCTION

The pathophysiology of TBI begins with the initial brain trauma (the primary injury). This primary injury results from mechanical damage that disrupts the blood-brain barrier (BBB), alters the vasculature and damages brain tissue (Dugue, 2017; Raheja, 2016). The resulting injured glia and neurons release their intracellular contents into the extracellular space and activate neighboring glia and neurons. Activated glia and neurons then produce molecular signals that can both exacerbate and mend the acute injury and contribute to long- term recovery (Dugue, 2017; Raheja, 2016). Secondary injury in TBI involves a host of molecular and cellular responses to the primary impact including: an influx of peripheral inflammatory cells through the disruptedBBB leading to the release of reactive oxygen species (ROS), cytokines, chemokines, and freeradicals; the excessive release of excitatory neurotransmitters in response to ion imbalanceacross the cell membrane following adenosine triphosphate (ATP) depletion and metabolic dysregulation and significant increases in intracellular calcium concentration that contribute to protease, nuclease and lipase/phosphatase activation (Dugue, 2017). All of these factors culminate in cellular dysfunction and cell death/loss via rapid (necrotic) and more delayed cell death pathways (apoptotic). These downstream molecular and cellular processes (the secondary injury) are the focus of many pre-clinical and clinical therapeutic studies (Dugue, 2017; Raheja, 2016; Townend, 2002). The spatiotemporal distribution of S-100B, IL-6, AQP4 production in secondary injury are some key marker acute feature after TBI pathophysiology. By examining changes of individual this protein, we can expect to identify novel approaches to TBI intervention/therapy (Dugue, 2017; Townend, 2002).

Hypotheticly, post-traumatic suppression of the hypothalamic- pituitary-adrenal axis and an increase in cytokine-mediated peripheral aromatase activity, leading to an imbalance in sex steroid estrogen, progesterone, and testosterone serum levels, have been areas of active interest for precise understanding of that mechanisms involved. (Raheja, 2016) Research of progesterone had been showed failed until phase III trial, however many reason for more investigation for this potential therapy in TBI (Raheja, 2016; Cutler, 2005; Cutler, 2007; Schumacher, 2007; Stein, 2008; Stein, 2008; Farace, 2000; Wright, 2001; Djebaili, 2005; Yao, 2005; Chen, 2007; Wright, 2006; Gibson, 2008; Gibson, 2009; Xiao, 2008; Verkman, 2008; Taya, 2010; Fukuda, 2012; Cheng, 2019). According to the recent literature deals with these biomarkers in isolation, and there is a paucity of existing literature for establishing a comprehensive model dealing with all 3 domains biomarker S-100B, IL-6, AQP4. To this end,analyzed serum biomarkers (S100-B, IL-6, AQP4) as part of a randomized placebo-controlled of progesterone in patients with severe TBI (sTBI), and analyzed the long-term predictive value of these biomarkers on the dichotomized Glasgow Outcome Scale (GOS) score at 3 months (Townend, 2002; Cutler, 2005).

MATERIALS AND METHODS

Study design: This study was part of a prospective, outcome- assessor–and statistician-blinded, randomized, placebo- controlled trial of progesterone. The population included adult (age 15–60 years) patients with severe TBI (sTBI) patients (Glasgow Coma Scale (GCS) score 4–8, who presented at our care trauma center, Hospital Dr Sutomo Surabaya within 24 hours after injury. We obtained approval from our institutional ethics committee prior to commencement of the trial. Of 40 patients with sTBI who were randomized for the trial, we prospective serially analyzed 3 serum biomarkers S-100B, AQP4, and IL-6. We analyzed the long-term predictive value of serum biomarkers on dichotomized GOS score 3 months (poor recovery/ unfavourable, GOS 1–3; good recovery /favourable, GOS 4–5).

Biomarker and Intervention Assessment: The serum levels of S-100B (WKEA Med), IL-6 (WKEA Med), AQP4 (WKEA Med) were determined using a commercially available kit based on the principle of the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique. The samples for the determination of these biomarkers were taken at the day I (24 hours), immediately after randomization and before intervention gived treatment natural Progesterone (Shandong Yikang Pharmaceutical) 1 mg/kgBW single dose, intramuscular and then day IV (96 hours) later.

Statistical Analysis: Using IBM SPSS Statistic software version 22, the baseline parameters, change of serum biomarkers, and outcome are presented as either number (percentage), mean, or median wherever appropriate. Analysis for 24 hours, 96 hours, and average serum biomarkers stratified according to outcome (The dichotomized GOS) was performed. To define independent factors of predicting outcome, a binary logistic regression model was used.GOS 3 months maked two category: Poor outcome (value label 1) for GOS score 1 – 3 and good outcome (value label 2) for GOS 4 – 5. The impact of factors on outcome was expressedas the OR (95% CI). A receiver operating characteristic (ROC) curve was made for independent factors predicting outcome, along with estimation of area under the curve (AUC). In line with current statistical consensus, an AUC of 0.8–0.9 is consideredvery good, 0.7–0.8 is considered adequate, and < 0.7 is considered poor in terms of accuracy of the test underconsideration. To analysis the trend of biomarker for explained cellular or molecular scene just only saw the difference Δ mean value (96 hours minus 24 hours); (+) meaning increased, (-) meaning decreased.To compare GOS dichotomized on both groups using the parametric comparation Paired t Test. A p value of < 0.05 was considered significant.

RESULTS

Demographic Profile: A total of 39 patients (male (100%)) with mean age 23,8 years old (yo), with range 16 yo – 47 yo, were analyzed for the current study (see Table 1). 1 patient (2,5%) patients were lost to follow-up at 3 months, respectively.Unfavorable GOS scores were found 19 (82,6%) in control group and 13 (81,25%) in intervention group after enrollment. (see Table 2)

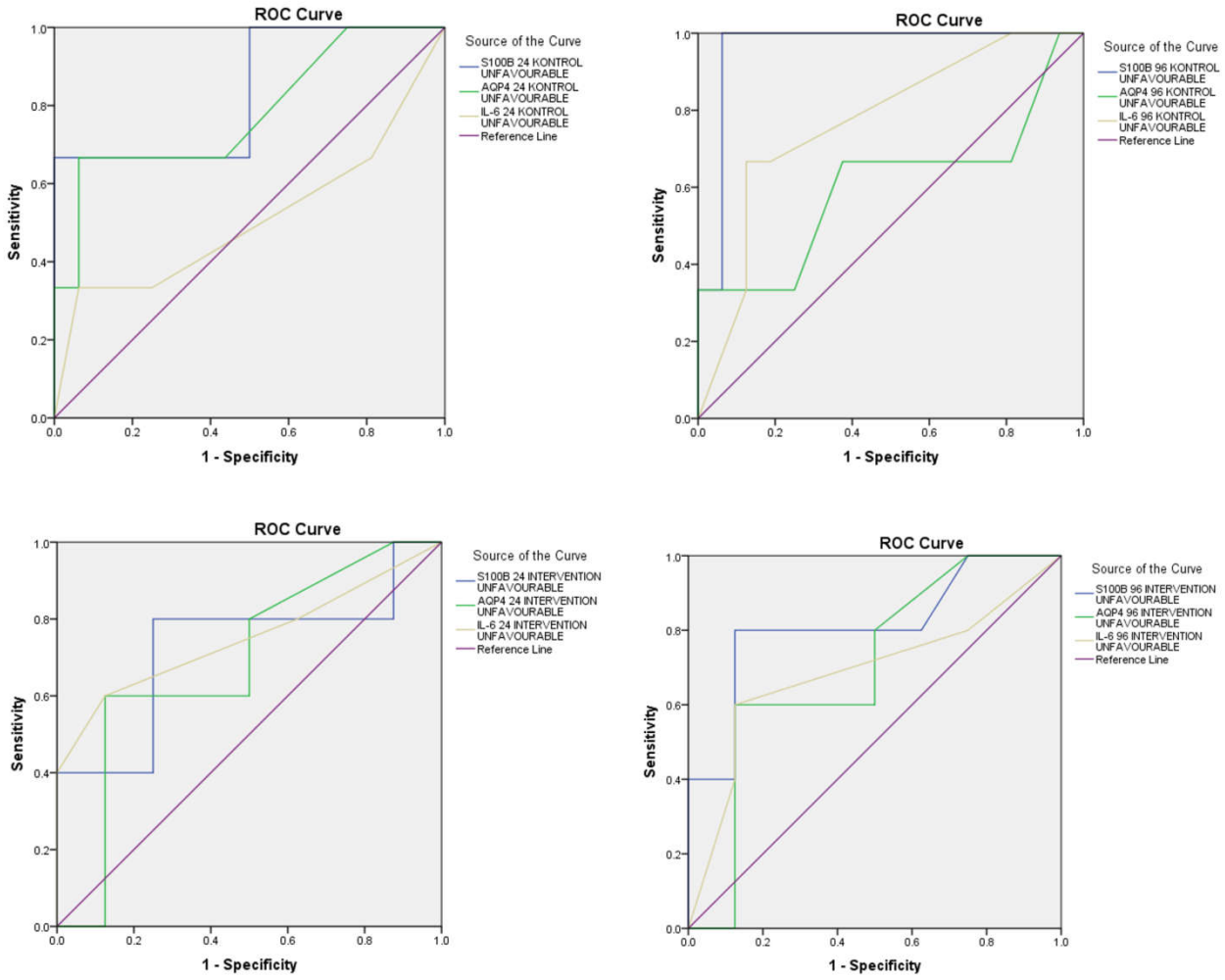
Analysis of Serum Biomarkers: prediction and change: The serum levels S-100B, AQP4 and IL-6 were across the dichotomized GOS groups at 3 months in both groups. GOS 3 months maked two category: Poor outcome (value label 1) for GOS score 1 – 3 and good outcome (value label 2) for GOS 4 – 5. Binary logistic regression result showed all value biomarker significant model to prediction the GOS dichotomy.At control group Initial -2 Log Likelihood: 21.254; Variable in equation df 1, sig.0.005; Cox & Snell R Square 0,603, Nagelkerke R Square 1.000, Hosmer and Lameshow

Table 1. Demographic profile

Characteristic	Intervention(N=16)	Control(N=23)
Age – yr	χ = 24,69	χ=23,26
Median	19	19,5
Age group – no.%		
< 20 years old	8 (50)	12 (52,17)
20 – 40 years old	7 (43,75)	10 (43,48)
>40 years old	1 (6,25)	1(4,35)
Total	16 (41)	23 (59)
Male Sex – no. %	16 (100)	23 (100)
Cause of injury – no.%		
Motorcycle accident	33 (84,6)	
Fall	6 (15,4)	

Table 2. The Difference (Δ mean values) on biomarker change day to day and comparative biomarkers outcome prediction value by paired t test (p)

Biomarker	Good Outcome GOS (4-5) 3 months			Poor Outcome GOS (1-3) 3 months		
	24 hours	96 hours	ΔX Value	24 hours	96 hours	ΔX Value
Control group	N= 4	N= 4		N= 19	N= 19	
S100B	25.67(p=0.031)	24.57 (0,031)	- 1,1	47,30 (0,000)	43,18 (0,000)	-4,12
AQP4	3.37 (p=0,02)	3,85 (0,000)	0,48	1.64 (0,000)	1.4 (0,001)	-0,24
IL-6	4,17 (p=0,000)	4,2(0,000)	0,03	1.98 (0,000)	2.01 (0,000)	0,03
Intervention group	N=3	N=3	ΔX Value	N=13	N=13	ΔX Value
S100B	36,16 (0,013)	33,2 (0,000)	-2,96	38.66 (0,000)	44.49 (0,000)	5,83
AQP4	3,7 (0,000)	3,7 (0,001)	0	1.14 (0,014)	1,01 (0,027)	-0,13
IL-6	0	0	0	1,87 (0,000)	1,84 (0,00)	-0,03



The ROC Curve Analysis (Fig.1)

Table 3. AUC result on predicts unfavourable outcome GOS 3 months dichotomy

Biomarker AUC	24 hours	96 hours
Control group		
S-100B	0,833	0,958
AQP4	0,781	0,604
IL-6	0,510	0,771
Intervention group		
S-100B	0,725	0,813
AQP4	0,688	0,700
IL-6	0,750	0,688

Table 4 .Result of Progesterone Analysis

Outcome (GOS 3 mt)	Progesterone (N=16)	Control (N=23)	Comparation Sig. (95% CI)
Primary efficacy analysis – no.%	χ = 2,38. SD=1,147	χ = 2,57. SD=1,037	P=0,580
Dead	5 (31,3)	3 (13,0)	
Vegetative state	3 (18,8)	9 (39,1)	P=0,819
Severe Disability	5 (31,3)	7 (30,4)	
Moderate Disability	3 (18,8)	3 (13,0)	P=0,423
Good Recovery	0	1 (4,3)	

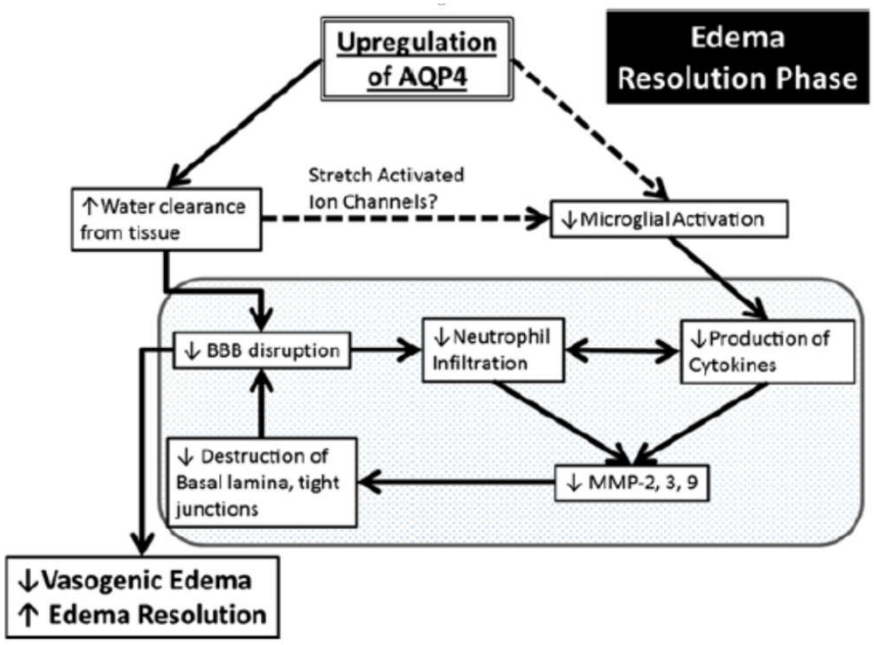


Figure 1. The potential link between AQP4, edema and neuroinflammation

At intervention group: initial -2 Log Likelihood: 15.442; Variable in equation df 1, sig 0,022, Cox & Snell R Square. 0.619, Nagelkerke R Square 1.000, Hosmer and Lameshow 0.000.

The difference mean value (Δx) 96 hours – 24 hours than analysis at control group and intervention group. (see.Table2). From Control group collected data analysis in prediction from good outcome to poor outcome (to right axis direction) we have the simulation equation:

S100B was increase:

Δunfav.= ΔX good - ΔX poor : -1,1 – (-4,12) = 3,02, AQP4 was decrease:
Δunfav.= ΔX good - ΔX poor : 0,48 – (-0,24) = 0,72 and IL-6 no change:
Δunfav.= ΔX good - ΔX poor : 0,03 – 0,03 = 0

From Intervention group collected data analysis in prediction from good to poor outcome (to right axis direction) we have the simulation equation:

S100B was extremely high decrease:

Δunfav.= ΔX good - ΔX poor: (-2,96) – 5,83 = (-8,79) AQP4 was less decrease compare to control:

Δunfav.= ΔX good - ΔX poor : 0 – (-0,13) = 0,13 IL-6 changes to decrease compare from to control:
Δunfav.= ΔX good - ΔX poor : 0 – (-0,03) = 0,03

According statistical consensus, an AUC of 0.8–0.9 is considered very good, 0.7–0.8 is considered adequate, and <0.7 is considered poor in terms of accuracy of the test under consideration. S-100B 24 hours and or 96 hours are the best or very good predicton for the unfavorable GOS score (1 - 3) at 3 months (AUC > 0,800).

Analysis of the outcome: There were no adverse event of Progesterone found in this research. Statistical analytic comparation study (compare mean – paired sample T Test) showed Intervention / Progesterone group had no significant value from control group. (p=0,580) within outcome scale GOS 3 months dichotomized.

More over there was no significant value in unfavourable (GOS score 1-3) outcome (p=0,819) outcome 3 months and favourable (GOS score 4-5) outcome 3 months (p=0,423).

DISCUSSION

In this research all of biomarker test is adequate and good accuracy for predicting. The best good accuracy is S-100B.

Brain Tissue Antigens (S-100B): A Ca^{2+}-binding protein ($t^{1/2}$ approximately 2 hours) predominantly secreted by astrocytes, S-100B is correlatedwith contusion volume (Dugue, 2017; Raheja, 2016; Townend, 2002). Despite a few studies showing serum S-100B as a predictor of outcome, its serum assay is ideal because of its not limited blood-brain barrier (BBB) permeability but short half-life (small window for sampling),and peripheral source of production (besides CNS) (Dugue, 2017; Raheja, 2016; Townend, 2002; Oliviera, 2008; Thelin, 2017; Thelin, 2017).Cerebrospinal fluid or brain tissue S-100B estimation is abetter predictor of outcome (Dugue, 2017; Raheja, 2016; Townend, 2002; Oliviera, 2008; Thelin, 2017; Thelin, 2017; Pham, 2010; Thelin, 2019; Tala, 2000). In this study S-100B have the best or good accuracy for predict the unfavourable and or favourable outcome (AUC 0,8 – 0,9). S-100B serum consistently for predict the outcome where decreased mean tend to good outcome and increased tend to poor outcome. From control group collected data: if wepredict favourable outcome to become unfavourableoutcome there wasincreasing S-100B. (ΔXunfav. = X good – X poor → -1,1 – (-4,12) = 3.02). In intervension group study we found consistently extreme result (ΔXfav. = Xpoor – Xgood = 5,83 – (-2,96) = 8,79. It presumed Progesterone effect can reduce S-100B 2 times or more to decreasing level of S-100B in secondary injury. However, Progesterone effect on dynamic of S-100B in severe TBI had unclearly concept theory in role of repairing neuronal injury and or BBB disruption. The other consideration is concept theory temporal trajectory of S100β that masked the effect of progesterone on S-100B as potential therapy. Independently S-100B only prediction the outcome, not modulating by progesterone effect.

Cytokines (IL-6): Interleukin-6 is cytokine expressed in CNS (microglia, astrocytes, and neurons). The existing literature regarding its roleas an independent predictor of Intra Cranial Pressure (ICP), mortality, and outcome (Dugue, 2017; Townend, 2002; Ndraha, 2010). It is detectable by 1hour postinjury and peaks in brain parenchyma at approximately2–8 hours[1]. Itinhibits Tumour Necroting Factor (TNF-α) synthesis and *N*- methyl-d-aspartate (NMDA)–mediatedtoxicity, and it induces Nerve Growth Factor (NGF) and promotes neural differentiation and survival (Dugue, 2017; Ndraha, 2010). Despiterepresenting BBB dysfunction and more reliable serumconcentrations than TNF-α, it is still less specific andpartly affected by the addition of polytrauma to the TBImodel. For increasing specificity in the TBI model, recentstudies have incorporated the NGF:IL-6 ratio as a morespecific predictor and have also included cerebral microdialysisfor the most accurate estimation of ongoing variationof cytokines in close vicinity to the actual lesion (Dugue, 2017). In this study IL-6 have adequate accuracy for predict the unfavourable and or favourable outcome (AUC 0,7 – 0,8). May be a factor above explained this IL-6 accuracy. From difference Δ simulation there was no changeon IL-6 study in control group. May be this limit explain IL-6 still adaptive to brain response after injury. In intervention group there was IL-6 changes to decrease compare from to control group: Δunfav = ΔX good - ΔX poor: 0 – (-0,03) = 0,03. It presumed progesterone mechanism was inhibited expression of proinflammatory genes. Elevation in these proinflammatory cytokines is an adaptive response of the brain to injury, which causes transientdestruction and apoptosis of damaged neural cells,paving the way for the reparative process (Dugue, 2017). Progesterone mediates its neuroprotection by reducing cerebral edema, lipid peroxidation, isoprostanes, andexpression of proinflammatory genes; generating metabolites that reduce pro-apoptotic and increase anti-apoptotic enzymes; and modifying the expression of vascular endothelial growth factor, brain-derived neurotrophic growth factor, and aquaporins responsible for development of edema (Van, 2006; Li, 2015). In inflamation research progesterone reported decreasing IL-6 after an injection of progesterone to model or disease.

Development of cerebral edema – brain water transporter (AQP4): AQP1 and AQP4 are most prevalent in CNS (Papadopoulos and Verkman, 2013). AQP1, involved in CSF secretion, is primarily expressed in the ventricular-facing plasmalemma of choroid plexus epithelium; it is absent from cerebrovascular endothelium (except in circumventricular locations lacking a BBB) (Papadopoulos and Verkman, 2013). AQP4 localizes to brain-fluid interfaces including perivascular astrocyte endfeet, glia limitans, basolateral membrane of ependymal cells and subependymal astrocyte processes. In Cytotoxic Edema (CytE), water enters the CNS through AQP4 on perivascular astrocyte foot-processes. In Vasogenic Edema (VasE), water is eliminated through AQP4 via different routes: astrocyte foot-processes intothe blood stream, subpial astrocyte processes and pial cells into subarachnoid CSF, and across subependymal astrocyte processes and ependymal endothelium into the ventricle, the glymphatic system (Filippidis et al., 2016; Hubbard et al., 2018; Iliff et al., 2014). In TBI, since there are contributions from both CytE and VasE, determining the overall contribution of AQP4 to edema formation versus elimination has been challenging and may be related to spatial/temporal expression patterns. TBI studies have shown AQP4 downregulation for up to 48 h after TBI (potentially coinciding with VasE), some of which occurs specifically in regions with BBB disruption (Cartagena et al., 2014; Ke et al., 2001; Kiening et al., 2002; Liu et al., 2015; Zhang et al., 2015).

Other studies have shown AQP4 upregulation coinciding with CytE development within 72 h (Lopez-Rodriguez et al., 2015; Lu et al., 2013; Taya et al., 2010). A study in murine closed head njury demonstrated that, while there was a global increase in cortical and striatal AQP4 expression (peak at 7 days), perivascular AQP4 expression was markedly reduced by day 3 (persisting until day 28) (Renet al., 2013). While not clearly noted, this suggests that changes in AQP4 localization/loss of polarization, while potentially worsening VasE (by limiting water clearance), may be a compensatory

mechanism to counteract/decrease CytE (Ren et al., 2013). A subsequent study in mild-CCI demonstrated that astrocytic foot-process edema was reduced in AQP4–/–mice (from decreased CytE); however, the effects were smaller than AQP4 deletion in models of pure CytE, likely a consequence of the decreased AQP4-dependent clearance of VasE (Yao et al., 2015). Increased AQP4 expression has been reported in human TBI tissues, and CSF levels are significantly higher in patients with severe TBI versus controls.

Further studies are warranted to evaluate the impact on edema (Hu et al., 2005; Lo Pizzo et al., 2013). The upregulation of AQP4 caused increased water clearance from the tissue, which in turn causes decreased BBB disruption because of decreased pressure, and there is less neutrophil infiltration and decreased pro-inflamatory cytokines. This cause decreased MMP production which possibly result in less destruction of the basal lamina and tight junctions, causes an even greater decrease of the BBB. In another pathway (dotted lines), the increased water clearance from the tissue and extracellular space causes changes in the osmotic pressure, changing the activation state of the stretch activated ion channels expressed in microglia, causing less microglial activation, thus causing decreased pro-inflammatory cytokine. The resulting decrease in BBB disruption / permeability leads to decreased vasogenic edema or better edema resolution. In this study AQP4 have poor to adequate accuracy for predict the unfavourable and or favourable outcome (AUC 0,6 – 0,8). We were simulated in control group there AQP4 was decrease if the cases become unfavourable: Δunfav. = ΔX good - ΔX poor : 0,48 – (-0,24) = 0,72. Paradoxicaly if AQP4 being upregulation it means good condition will done. Its consistently to theory there was potential link between AQP4, edema and neuroinflamation. Its can be explained there was no change on IL-6 study in control group. May be this limit explain IL-6 still adaptive to brain response after injury while AQP4 just working. In intervention group showed AQP4 was less decrease if the cases become unfavourable compare to control: Δunfav.= ΔX good - ΔX poor : 0 – (-0,13) = 0,13. It presumed Progesterone play a role modulating in AQP4 than follow neuroinflamation cascade to reduce cerebral edema.The protective effect of Progesterone may be related to the down-regulation of AQP-4 expression (Li, 2015; Stein, 2008).

Conclusion

Serial monitoring of S-100B, IL-6 and AQP4 serum levels could aid in prognostication in patients with sTBI and guide us to direct more resources toward such patients for optimal outcome. S-100B is the best good accuracy for predict outcome. A cause and effect relationship of these biomarkers to outcome needs to be further studied for better understanding of the pathophysiology in sTBI and for choosing potential therapeutic targets. Progesterone have an effect in change of S-100B serum level expression that involve in neuronal injury, and the process of cerebral edema (modulating AQP4 link to IL-6). However, in this study Progesterone had no benefit in overall clinical outcome (GOS dichotomy 3 months).

Ethical clearance: Research approval were obtained from the Ethics Committee of the Medical Faculty, University of Airlangga – Hospital Dr Soetomo, East Java, Indonesia. Every research subject has the right to know the results of the examination conducted on him.

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REFFERENCEs

Dugue R, Nath M, Dugue A, Barone FC. Roles of Pro- and Anti-inflammatory Cytokines in Traumatic Brain Injury and Acute Ischemic Stroke.

Raheja A, Sinha S, Samson N, Bhoi S, Subramanian, A, Sharma P, et al. Serum biomarkers as predictors of long-term outcome in severe traumatic brain injury: analysis from a randomized placebo-controlled Phase II clinical trial.J Neurosurg.2016; 125:631–641.

Townend W J, Guy M J, Pani MA, Martin B, Yates DW. Head injury outcome prediction in the emergency department: a role for protein S-100B? J Neurol Neurosurg Psychiatry 2002;73:542–546

Oliviera CO, Ikuta N, Regner A.Outcome biomarker following severe traumatic injury. Rev Bras Ter Intensiva.2008; vol.20, no.4, pp.411 – 21

Thelin EP, Zeiler FA, Ercole A, Mondello S, Büki A, Bellander B, et al. Serial sampling of serum protein biomarkers for monitoring human traumatic brain injury dynamics: a systematic review.Front. Neurol. 2017; 8:300.

Thelin EP & David W, Nelson & Bo-Michael Bellander : A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochir 159:209–225, 2017.

Pham N, Fazio V, Cucullo L, Teng Q, Biberthaler P, Bazarian JJ, et al: Extracranial Sources of S100B Do Not Affect Serum Levels. PLoS ONE www.plosone.org , Volume 5, Issue 9, e12691, 2010

Thelin E, Faiez AN, Frostell A, Zetterberg H,Blennow K, Nystro¨m H, et al. A Serum Protein Biomarker Panel Improves Outcome Prediction in Human Traumatic Brain Injury. Journal of neurotrauma 36. Mary Ann Liebert, Inc. 2019 :2850–2862

Tala MI, Darmadipura S. Hubungan perubahan kadar protein S-100β dalam cairan serebrospinalis dengan tingkat kesadaran pasien cedera otak berat. Karya Akhir S2 skripsi, Airlangga University-School of Medicine,Surabaya, Indonesia, 2008

Ndraha E, Bajamal AH. Hubungan antara perubahan kadar S-100β pada pasien cedera kepala berat pada fase akut dengan outcome. Karya akhir S2 skripsi, Airlangga University-School of Medicine,Surabaya, Indonesia, 2010.

Benjamin A, Matthew L, Hitomi, E, Peng W, Liao Y, Lou N, et al:Biomarkers of Traumatic Injury Are Transported from Brain to Blood via the Glymphatic System. The Journal of Neuroscience, January 14, 35(2):518 –526, 2015.

Hergenroeder GW, Moore AN, Mc Coy JP, Samsel L, Ward NH, Clifton GL, et all. Serum Il-6 : a candidate biomarker for intracranial presure elevation following isolated traumatic brain injury.Journal of Neuroinflammation. 2010; 7:19.

Jhaa RM, Kochaneka PM, Simardf JM. Pathophysiology and treatment of cerebral edema in traumatic brain injury. Neuropharmacology 145. 2019: 230–246

Van Landingham JW, Cutler SM, Cekik M, Wright D, Stein DG. Serum biomarker profiling for progesterone treatment

of traumatic brain injury : a comparative study human and rat. FASEB Journal. 2006

Li X, Bai R, and Zhang J. Effect of progesterone intervention on the dynamic changes of AQP-4 in hypoxic-ischaemic brain damage. Int J Clin Exp Med. 2015;8(10):18831-18836

Cutler, SM : The effect of progesterone withdrawal on behavioral and molecular indices after traumatic brain injury, PhD thesis, Georgia Institute of Technology, Georgia, 2005.

Cutler SM, Milos C, Miller DM, Wali B, Vanlandingham JW, Stein DG. Progesterone improves acute recovery after traumatic brain injury in the aged rat, Journal of Neurotrauma. 2007; vol. 24, pp. 1475–86.

Schumacher M, Guennoun R, Ghoumari A, Massaad C, Robert F. Novel perspectives for progesterone in hormone replacement therapy, with special reference to the nervous system. Endocrine Reviews.2007; vol. 28, no.4, pp.387–439.

Stein DG. 2008. Progesterone exerts neuroprotective effects after brain injury. *Brain research reviews.* 2008; vol.57, pp. 386-97.

Stein DG, Wright DW, Kellermann AL. Does Progesterone have neuroprotective properties?. Annals of Emergency Medicine. 2008; vol. 51, no.2, pp.164-72.

Farace E, Alves WM. Do women fare worse? A metaanalysis of gender differences in outcome after traumatic brain injury. J. Neurosurg. 2000; vol. 93, pp. 539 – 45.

Wright DW, Bauer ME, Hoffman SW,Stein DG. Serum progesterone levels correlate with decreased cerebral edema after traumatic brain injury in male rats. Journal of Neurotrauma. 2001; vol. 18, no.9, pp. 901-9.

Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG.The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. Journal Of Neurotrauma. 2005; vol. 22, no. 1, pp. 106–18.

Yao XL, Liu J, Lee E, Ling GSF, Mccabe JT. Progesterone differentially regulates pro- and anti-apoptotic gene expression in cerebral cortex following traumatic brain injury in rats. Journal of Neurotrauma. 2005; vol. 22, no. 6, pp. 656–68.

Chen G, Shi J, Ding Y, Yin H, Hang C. Progesterone prevents traumatic brain injury-induced intestinal nuclear factor kappa b activation and proinflammatory cytokines expression in male rats.Hindawi Publishing Corporation Mediators of Inflammation. 2007;Article ID 93431, 7 pages.

Wright DW, Kellermann AL, Hertzberg VS, Clark PL,Frankel M : proTECT : A randomized clinical trial of Progesterone for acute traumatic brain injury. Annals of Emergency Medicine. 2006; vol. xx, no x, pp.1 – 13.

Gibson CL, Gray LJ, Bath PMW, Murphy SP. Progesterone for the treatment of experimental brain injury; a systematic review. Brain. 2008; vol. 131, pp. 318-28.

Gibson CL, Coomber B, Rathbone J. Is Progesterone a candidate neuroprotectivefactor for treatment following ischemic stroke?. The Neuroscientist.2009; vol. 15, no. 4, pp. 324-32.

Xiao G ,Wei J, Yan W, Wang W, Lu Z. Improved outcomes from the administration of progesterone for patients with acute severe traumatic brain injury: a randomized controlled trial. Critical Care. 2008; vol. 12,no 2, pp.1 – 10.

Verkman AS.ReviewAquaporins. translating bench research to human disease.The Journal of Experimental Biology.2008; vol. 212, pp. 1707-15

Taya K, Marmarou CR, Okuno K, Prieto R, Marmarou A. Effect of Secondary Insults upon Aquaporin-4 WaterChannels following Experimental Cortical Contusion in Rats. Journal of neurotrauma 27. 2010 :229–239.

Fukuda AM, and Badaut J. Aquaporin 4: a player in cerebral edema and Neuroinflammation. Journal of Neuroinflammation. 2012 : 9:279

Cheng, Y,Yeung, W L,Zhang, P D,Li, N,Kiang, M Y, et al.. Progesterone Is MoreEffective Than Dexamethasone in Prolonging Overall Survival and Preserving Neurologic Function in Experimental Animals with Orthotopic Glioblastoma Allografts. World Neurosurgery, Volume 125 – May 1, 2019

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