

Tryptophan Metabolism and Birth Asphyxia: what Implications for Neuro Development?

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In children, perinatal asphyxia remains a frequent cause of disability and death. Increase catabolism of tryptophan through the kynurenine pathway, occurs in human brain and systemic tissues alongside immune activation. The aim of this study was to determine the interaction between changes of the tryptophan pathway as well as cerebral and systemic inflammation triggered in asphyx-ic neonates and correlate these molecular changes with clinical parameters of asphyxia. The levels of the tryptophan catabolites, kynurenine and quinolinic acid, as well as cytokines were quantified in CSF and plasma of asphyx-ic neonates at 0 and 7 days after birth. Since macrophages and microglial cells are the source of quinolinic acid, we also measured chitotriosidase activity, which is a marker for monocytic activation. Significantly higher concentrations of IL-4, IL-6, IL-8, and IL-10 and a non-significant increase of TNF, IFN- γ were found in CSF of asphyxiated infants at day 1 compared to day 7. Most of the inflammatory parameters normalized at 7 days, but chitotriosidase activity remained elevated. The children were followed-up for an average of a 4-5 years period, and only in one case, the evaluation of general movements showed an absent fidgety.

1. Introduction

Severe asphyxia during birth is a common event in both term and pre-term neonates. This insult causes irreversible brain damage with long lasting physical disabilities and mental illnesses [1].

Neuroinflammation is one of the major responses elicited by perinatal asphyxia likely contributing to delayed and aggravated neural injury [2-6].

The inflammatory cascade comprises the secretion of several immune mediators, which can alter other molecular pathways. Recent work has shown that pro-inflammatory cytokines enhance the metabolism of the amino acid tryptophan [TRP], via the kynurenine pathway [KP]. Interferon [IFN]- γ in particular has been reported to be a key regulator of the KP [7]. Conversely, studies have shown that TRP plays an important role in regulating immune activation. In fact, decrease in TRP levels suppresses the proliferation of blood mononuclear cells [8] and inhibits allogeneic T-cell responses [9, 10]. Tryptophan is an essential amino acid required for protein synthesis in all living cells. In blood TRP is normally transported across the BBB into the brain by a sodium-independent amino acid transport system. TRP is metabolised into kynurenine [KYN] by indoleamine 2,3-dioxygenase [IDO-1] in astrocytes, infiltrated macrophages, microglia, dendritic and mast cells. KYN is then further processed into other neuroactive metabolites such as kynurenine acid [KYNA], anthranilic acid [AA], 3-hydroxykynurenine [3HK], 3-hydroxyanthranilic acid [3HAA], quinolinic acid [QUIN] and nicotinic acid [NA] [11]. QUIN is a key end product of the KP that acts as a N-methyl-D-aspartate [NMDA] receptor agonist, a known mediator of excitotoxic injury

and neuronal death, inducing lipid peroxidation, axonal damage and astrocytic death via various mechanisms [12]. In the brain, QUIN is highly produced by infiltrating macrophages and activated microglia and is neurotoxic at concentrations as low as 100 nM. Because QUIN depolarises neurons and astrocytes, it also plays a pivotal role in the onset of post-traumatic seizures, which can arise as a consequence of brain hypoxia [13, 14]. These findings have implicated QUIN in a variety of central nervous system [CNS] disorders [15-19] with its elevation being associated with rapid death in subjects with brain injury [20].

Another important product of the KP is the neuroprotective molecule KYNA. By being a NMDA receptor antagonist, QUIN and KYNA have opposite actions. Under normal condition, the levels of QUIN and KYNA are found in an equilibrium state that does not exhibit any detrimental effects on brain function. However, in CNS diseases QUIN is produced at higher concentration than KYNA. This excess in QUIN drives TRP metabolism towards neurotoxicity [21]. Studies have shown that macrophages are one of the major sources of QUIN production in the brain [22]. Abundant macrophage infiltration in the brain is a critical element of immune activation following asphyxia [23]. The accumulation of macrophages is easy to detect in the laboratory environment by standard histology, but it is more challenging to investigate in the *in vivo* settings, in particular in humans. Therefore, specific products of macrophage activation can function as surrogate markers of their brain accumulation. The human chitotriosidase [Chit] belongs to the glycoside hydrolase family 18 and is highly secreted by fully activated mononuclear cells in all tissues and, to a lesser extent, by peripheral polymorphonuclear leukocytes [20, 24]. Chit activity has been identified as a marker for macrophage activation in infectious diseases [25], neuroinflammatory diseases [26] and stroke [27].

The aim of this study was to determine the interaction between the neurotoxic changes elicited with the alteration of the KP and immune activation occurring in the brain of neonates suffering from perinatal asphyxia. Levels of TRP metabolites as well as immune mediators and parameter of macrophage activation were measured in matching CSF and plasma samples at 1 and 7 days after birth, and their levels correlated with biochemical parameters of asphyxia. The results were also correlated with long-term neurological scores to explore the potential of QUIN as a prognostic marker of brain damage and poor outcome in asphyxic children.

2. Material and Methods

2.1. Patients and Sample Collection

This study included 6 neonates admitted to the NICU [Neonatal Intensive Care Unit] of the University of Catania Hospital, Italy. All neonates were singleton and born via vaginal delivery at term gestation [37 to 41 weeks]. The body weights at birth were between 2400 to 3900 grams. All 6 babies have shown clear evi-

dences of severe birth asphyxia and required resuscitation [IPPV [intermittent positive pressure ventilation] and nCPAP [nasal continuous positive airway pressure]] for more than 1 min immediately after birth coinciding with Apgar scores <5 at 5 min after birth.

Systemic blood pressure and heart rate were monitored throughout the admission at the NICU. Within 6 h following admission, arterial blood was taken for measurement of pH, lactic acid, Ht [haematocrit], pCO₂, O₂ saturation, and FiCO₂ [fraction of inspired CO₂]. Lumbar puncture was performed to obtain CSF for screening of meningitis infection. The leftover CSF samples following clinical tests were kept at -70°C until the measurement of cytokines, Chit activity and TRP metabolites. Lumbar puncture was repeated at day 7 and measurements were conducted as indicated above. This study had received prior approval from the institutional human ethics committee and written consents were obtained from the parents.

2.2. Multiplex Assay for Cytokines

The concentration of IL-6, IL-8, IL-10, IFN- γ , and TNF was determined using a Bio-Plex Cytokine Assay System [Bio-Rad, Sydney, Australia] in CSF according to the manufacturer instructions. In brief, samples [50 μ l] or standard and a mixture of cytokine antibody-coupled beads [50 μ l] were added into a 96-well filter plate in duplicates, mixed and incubated overnight at 4°C. After removal of the incubation buffer and washing, a cocktail of biotinylated antibodies [against IL-6, IL-8, IL-10, IFN- γ , and TNF] was added in each well to react with cytokines. Fluorescent-tagged streptavidin [streptavidin-phycoerythrin] was then added to forming a complex of bead-cytokine-antibody-fluorescent. The concentration of each cytokine was determined by a Bio-Plex Suspension Array System [Bio-Rad] that quantifies cytokines based on the different colour of beads representing different cytokines and the presence of streptavidin-phycoerythrin. Cytokine concentrations were automatically calculated by the Bio-Plex Manager software [Bio-Rad] using a standard curve derived from recombinant cytokine standards.

2.3. Chitotriosidase Activity Assay

Measurement of Chit activity was performed using enzyme activity assay described previously [28]. Briefly, 5 μ l of undiluted plasma or 30 μ l of CSF were diluted with 100 μ l solution containing 22 mM chitotriosidase substrate 4-methylumbelliferyl-beta-D-N,N,N-triacetyl-chitotriose [Sigma Chemical Co.] and 0.5 M citrate phosphate buffer pH 5.2 for 15 min at 37°C, as originally described by Hollak et al [29]. The reaction was stopped with the addition of 2 ml of 0.5 M Na₂CO₃-NaHCO₃ buffer pH 10.7, and the product 4-methylumbelliferone was read on a Hitachi 2500 fluorimeter at excitation 365 nm and emissions 450 nm. Chit activity was expressed as nanomoles of substrate hydrolyzed per millilitre per hour [nmol/ml/h] against standards. Chit activity was repeated in at least two assays for each sample to obtain media/

mean values.

2.4. Measurement of Tryptophan Metabolites

TRY, KYN, KYNA and 3HAA were quantified using high performance liquid chromatography [HPLC]. CSF and serum samples were extracted using equal volume of 5% trichloroacetic acid and centrifuged at 2000xg for 15 min to pellet the precipitates. The supernatants were then removed, filtered before being injected into HPLC system. The HPLC consists of a solvent delivery pump [GBC LC1110, Australia], an auto-sampler [Shimadzu SIL-10AD, Japan], a solvent degasser [Shimadzu DGU-14A] and a WinChrom chromatography data acquisition system [GBC, Australia]. For TRY and KYN, the mobile phase consisted of sodium acetate [40 mM] and citric acid [40 mM] adjusted to pH 5 before the addition of acetonitrile to a final concentration of 2.5%. This was passed through a 0.45 µm filter [Millipore] using a pump at a flow rate of 1 ml/min. Fifty µl of extracted supernatant or standard were injected into the HPLC, the metabolites separated by a C18 column [Onyx Monolithic, Phenomenex, Australia] and quantified using a photodiode array detector [Shimadzu SPD-M10A] at absorbance of 278 and 363 nm for TRP and KYN, respectively. KYNA was measured using the same HPLC setup described above except that the detection was performed by a fluorescence detector [GBC] at excitation of 344 nm and emission at 388 nm, a polymeric column [20 × 3.2 mm; 5 µm particle size; Polymer Lab] and the mobile phase consisted of sodium acetate [50 mM], zinc acetate [0.25 M] and 5% acetonitrile with pH 6.3 at the flow rate of 1 ml/min. 3HAA was measured by a Synergi Hydro column [250 × 4.60 mm; 4 µm particle size; Phenomenex] connected to a fluorescent detector [GBC, Ex = 320 nm, Em = 420 nm] with mobile phase containing of 25 mM sodium acetate and 2.5% acetonitrile, pH 5.5 and a C18 column.

QUIN was quantified by gas chromatography–mass spectrometry [GC-MS]. The extracted supernatant of CSF was prepared following the procedures described previously [30]. GC-MS was carried out by an Agilent Technologies 6890 gas chromatograph, an Agilent 5973 mass selective detector [Agilent Technologies, Sydney, Australia], a HP-5MS capillary column, a GC oven, and a 7683

autosampler [Agilent Technologies]. Finally, QUIN concentration was determined as dihexafluoroisopropyl ester, a derivative of QUIN.

2.5. Assessment of Metabolic Status

The concentrations of glucose, lactate, glycerol and glutamate in CSF were measured by an ISCUS analyser [CMA Microdialysis, Sweden] against the standards provided by the manufacturer.

2.6. Data Analysis

Since the concentration of cytokines, TRP metabolites, glucose, lactate, glycerol, glutamate and the activity Chit were not normally distributed. A Mann Whitney U Test was used for graphic representation and statistical analysis. Data were shown as dot plots for each individual value and presented as median with interquartile range for comparison, $p < 0.05$ considered significant.

3. Results

3.1. Clinical Physiological Data

The clinical observations data of these asphyxiated neonates enrolled in the study are summarised in [Table 1]. The children were followed-up for an average of 4-5 years period [range 2.5-8 years]. Patient #2 showed an IPV in the basal nuclei by echography [data not shown] while the evaluation of general movements showed an absent fidgety. In this neonate, the sitting was observed at 11 months and the walking at 24 months. Bayley Scales of Infant Development was determined at 65 at 24 months. While the general movement in some infants showed a normal pattern [Patients #1, #4, #5], in other newborns [Patients #2, #3 and #6] the general movement demonstrated a poor repertoire with absent fidgety. The ability to sit occurred at 8 months in five asphyxiated newborns [#1, #3, #4 and #6], while in one case [#2] it was first manifest at 11 months. All children presented a marked delay in walking ability namely at 14 months [#5], 17 months [#1, #4, #6] and 19 and 24 months [#3 and #2 respectively]. Only one newborn received breast milk and his Q.I ranged between 62-100 according the other development parameters. Biochemical parameters reflecting anaerobic metabolism were confirmed by low blood O₂ saturation [$< 90\%$] and pH [< 7.00] and high lactate [> 9 mM] in blood. These and other parameters are summarized in [Table 2].

Table 1: Clinical data and neurological follow-up of asphyxiated patients.

Patients	Gestational Age at Delivery (weeks)	Birth Weight (g)	Breast Milk	Bilirubin Day 1	Bilirubin Day 2	Sitting (months)	Walking (months)	Q.I. (Bayley)	Echography
#1	38	2800	yes	1	0.6	8	17	87	IPV diffuse
#2	37	2400	no	1.3	4	11	24	65	IPV basal nuclei
#3	39	3900	no	1.4	3.2	8	19	62	IPV diffuse
#4	39	2660	no	3.9	8.4	8	17	68	Normal
#5	41	3360	no	3	5.3	9	14	100	IPV moderate
#6	40	3200	no	1.3	4	8	17	80	Normal

Table 2: Clinical and biochemical data of 6 neonates considered asphyctic at birth

Patients	Day	pH	Lactate (mM)	Hematocrit (%)	PaCO ₂ (mmHg)	Sat O ₂ (%)	FiCO ₂ (%)	HR (beats/min)	Arterial Pressure (mmHg)
#1	0	7.1	9.7	61.3	35.3	91	50	108	65/44
#1	7	7.4	1.4	54	46	95	21	130	64/35
#2	0	7	11.8	61	66.7	91	50	154	84/58
#2	7	7.4	1.2	42	38	95	21	130	64/35
#3	0	6.8	14	60	127	65	90	75	68/29
#3	7	7.4	1.2	42	38	95	21	130	64/35
#4	0	6.9	9	66	70	65	90	75	68/29
#4	7	7.4	1.9	49	38	95	21	130	64/35
#5	0	6.9	9.4	52.6	48.2	91	50	120	56/27
#5	7	7.4	1.1	54	35	94	21	140	56/35
#6	0	7.26	2.3	64.3	58.9	96	30	160	56/24
#6	7	7.4	1.4	54	46	95	21	130	64/35

pCO₂: partial pressure of carbon dioxide; sO₂: arterial O₂ saturation; FiCO₂: fractional concentration of inspired CO₂; HR: heart rate.

3.2. Cytokine concentration in CSF of Asphyctic Neonates

Following birth asphyxia, six cytokines [IL-4, IL-6, IL-8, IL-10, IFN- γ , and TNF] were quantified in CSF at 1 and 7 days after birth [Table 3]. Amongst them only IL-8 displayed marked changes in all infants with a significant elevation at day 1 followed by a sub-

stantial reduction at day 7 [median: 594.1 pg/ml, IQR: 256.2-707.5 pg/ml; median 41.68 pg/ml, IQR: 31.74-70.51 pg/ml; respectively; p<0.05]. IL-6, TNF and IFN- γ were not increased in CSF after asphyxia at either day with the exception of patient #1 who had much higher levels of cytokine concentrations than other patients.

Table 3: Cytokine concentrations in cerebrospinal fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Cytokines (pg/ml)	CSF Day 1	CSF Day 7	Serum Day 1	Serum Day 7
IL-4	0.068 \pm 0.063 (0.926)	n.d.	0.030 \pm 0.016 (0.533)	0.183 \pm 0.155 (0.846)
IL-6	242.58 \pm 177.68 (0.732)	10.32 \pm 5.43 (0.526)	182.34 \pm 29.39 (0.161)	34.88 \pm 15.59 (0.446)
IL-8	632.03 \pm 153.43 (0.242)	49.23 \pm 10.30 (0.209)	169.03 \pm 92.55 (0.546)	24.74 \pm 5.50 (0.222)
IL-10	22.47 \pm 10.83 (0.481)	0.90 \pm 0.45 (0.5)	1697.50 \pm 579.8 (0.341)	62.95 \pm 33.54 (0.532)
IFN- γ	18.58 \pm 9.97 (0.536)	2.47 \pm 0.55 (0.222)	16.33 \pm 3.78 (0.231)	11.90 \pm 7.03 (0.590)
TNF- α	3.59 \pm 2.51 (0.699)	0.62 \pm 0.12 (0.193)	4.10 \pm 0.79 (0.192)	6.15 \pm 4.06 (0.660)

coefficient of variation σ/μ in brackets; n.d.: not detectable

3.3. Evaluation of CSF Energy Metabolism in Asphyctic Neonates

[Table 4] reports values of different metabolic molecules evaluated in CSF and serum of all asphyctic neonates. Glucose had similar concentrations in CSF between day 1 and 7, and these values are similar to glucose levels in serum of normal babies [31]. In contrast, lactate was significantly higher in the CSF at day 1 [median: 6654 μ M, IQR: 1825-7959 μ M] as compared with day 7 [median: 1126 μ M, IQR: 932.6-1255 μ M; Fig 2 B]. Instead, significant higher glycerol concentration was observed in the CSF at day 1 after birth [median: 192 μ M, IQR: 74-233 μ M], then decreased

significantly at day 7 [median: 20 μ M, IQR: 15.5-56.5 μ M] indicating higher brain cell membrane damage at day 1. There was no change in CSF glutamate levels between day 1 and 7, which has no association with potential cell membrane damage. CSF glycerol concentration was high on day 1 [195 \pm 41 μ M] and returned to almost normal level by day 7 [32.8 \pm 10.2 μ M]. CSF glutamate increased at day 1 after birth as compared with day 7. There was no difference in serum glycerol levels between day 1 and 7, while serum glutamate showed elevated concentration in day 1 after birth. CSF and serum lactate and glucose appeared no changes between the samples from 2 days.

Table 4: Metabolite concentrations in cerebrospinal fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Metabolites (μM)	CSF Day 1	CSF Day 7	Serum Day 1	Serum Day 7
Glucose	2647.8 \pm 455.7 (0.172)	1914.3 \pm 448.4 (0.234)	3043.3 \pm 683.0 (0.224)	3599.7 \pm 217.0 (0.060)
Lactate	6287.7 \pm 1238.9 (0.197)	1100.2 \pm 71.3 (0.647)	7194.0 \pm 1357.7 (0.188)	2425.6 \pm 824.9 (0.340)
Pyruvate	18.2 \pm 5.3 (0.291)	8.0 \pm 1.8 (0.225)	77.8 \pm 10.4 (0.133)	40.7 \pm 18.2 (0.447)
Glycerol	195.0 \pm 41.9 (0.214)	32.8 \pm 10.2 (0.310)	93.2 \pm 11.9 (0.127)	83.8 \pm 21.1 (0.251)
Glutamate	5.6 \pm 0.9 (0.160)	1.7 \pm 0.4 (0.235)	147.3 \pm 32.6 (0.221)	164.1 \pm 39.7 (0.241)
L/P ratio	552.7 \pm 269.6 (0.487)	666.0 \pm 442.5 (0.664)	89.8 \pm 14.0 (0.155)	77.7 \pm 16.6 (0.213)

coefficient of variation σ/μ in brackets.

3.4. Tryptophan Metabolism

The median concentration of TRP in CSF at day 1 was lower than day 7 [0.92 μM vs 3.60 μM], however, the variability of the TRP concentration was quite large among different infants [IQR: 0.74-7.21 μM], as compared with day 7 [0.49-4.67 μM]. The significant decrease of KYN in CSF at day 7 as compared with day 1 was

not associated with changes of other downstream metabolites [i.e. 3HAA, KYNA and QUIN] over time [Table 5].

3.5. Chitotriosidase Activity in CSF

The median Chit plasma level in asphyxial newborn was 9.86 nmol/mL/h [IQR: 6.61-13.88 nmol/mL/h] and 5.57 nmol/mL/h at day 7 [IQR: 4.39-8.24 nmol/mL/h], the difference did not reach the significant level [Mann Whitney U test, $p=0.082$; Table VI].

Table 5: Tryptophan, and kynurenine metabolite concentrations in cerebrospinal fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Tryptophan and Kynurenine Metabolites (nM)	CSF Day 1	CSF Day 7	Serum Day 1	Serum Day 7
TRY	3657.2 \pm 1521.1 (0.416)	2741.0 \pm 864.9 (0.315)	5231.4 \pm 620.1 (0.118)	5474.2 \pm 524.0 (0.095)
KYN	553.8 \pm 65.6 (0.118)	463.6 \pm 65.6 (0.141)	5074.8 \pm 1222.8 (0.240)	4900 \pm 775.7 (0.158)
KYNA	95.7 \pm 17.9 (0.187)	97.6 \pm 38.3 (0.392)	743.7 \pm 187.1 (0.251)	227.5 \pm 69.7 (0.306)
3OH-AA	1176.3 \pm 195.4 (0.166)	1098.8 \pm 205.5 (0.187)	1425.5 \pm 122.4 (0.085)	148.7 \pm 97.7 (0.657)
QUIN	205.5 \pm 64.4 (0.313)	132.2 \pm 38.1 (0.288)	-	-

coefficient of variation σ/μ in brackets.

Table 6: Chit activity in cerebrospinal fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Chit activity	CSF		Serum	
	Day 1	Day 7	Day 1	Day 7
Chit	9.19 \pm 7.47	6.64 \pm 2.67	11.21 \pm 6.11	12.98 \pm 7.57

4. Discussion

Increasing evidences have demonstrated significant role of cytokine in the brain as injury mediators in animal models [32-34], as predictive biomarkers in neurodegenerative diseases [35-38] and inflammation-driven depressive disease [16-19], in adult brain injury [39] as well as neonatal brain injury [40]. The metabolism of tryptophan plays a relevant role in the production of neurotoxic substance but in the same time of neuroprotective substances [16]. This is a surprising fact but the different link of these two products to receptor of glutamate justify the bivalent function in the brain. There are some recent studies where the control of this mechanism

was proposed utilizing in experimental model the effect of adenosine A2A receptor antagonist, an inhibitor of quinolinic activity [41], or the effect kinurenine 3-hydroxylase inhibitor in rodents potentiate the expression of kinurenic acid [42], providing neuroprotection in neonatal rodents. However, the clinical use of this inhibitors did not receive sufficiently guaranties to be proposed in conditions where the quinolinic acid is increases. One of this application could be the asphyctic newborns, subjects with brain trauma, stroke and other degenerative brain injury. In this context, the vulnerability-stress-inflammation model has been supported by growing evidence [16-19]. According to the model, genetic land-

scape predisposes subjects to be easily affected by stress, which would show inflammatory responses later in life [16]. In similar way, prenatal maternal stress [e.g., prenatal infection/inflammation, decreased fetal growth, hypoxia-related obstetric complications] has been linked to the development of CNS disease in offspring [43].

This study revealed that asphyxial newborns have a significant increase of proinflammatory cytokines in both CSF and in plasma and that this increase was more significant in CSF likely due to the presence of activated macrophage and glial cells. The normalization of these immunological parameters was demonstrated in 7 days, but the levels of Chit activity remained high due to the persistence of macrophage and glial cells activation with the function of sweeper or scavenger of detrimental material. In relation to the macrophage activity the quinolinic acid was found significantly increased in CSF respect plasma and this neurotoxic substance was maintained over the 7 days of observation especially in CSF. This observation is not surprising because a persistent increase of quinolinic acid was reported in the CSF of subject with chronic brain trauma dating a year prior [44].

Within minutes of a traumatic impact, a robust inflammatory response is elicited in the injured brain. The complexity of this post-traumatic sequel involves a strong cellular response comprising the activation of resident microglia and astrocytes, as well as the infiltration of blood leukocytes. The second component regards the secretion of immune mediators, which can be divided into the following sub-groups: the archetypal pro-inflammatory cytokines [IL-1, TNF- α , IL-6], the anti-inflammatory cytokines [IL-4, IL-10, and TGF- β 1], and the chemotactic cytokines or chemokines, which specifically drive the accumulation of parenchymal and peripheral immune cells in the injured brain regions [45]. Whilst the humoral immune response is particularly pronounced in the acute phase following Traumatic Brain Injury [TBI], the activation of glial cells seems to be a rather prolonged effect lasting for several months [46]. The interactions between cytokines/chemokines and the various cell types triggers a complex network of events, which subsequently intersect with adjacent pathological cascades including oxidative stress, excitotoxicity, or neuroprotective and reparative systems including angiogenesis, scarring and neurogenesis [47]. It is well accepted that neuroinflammation is responsible of both beneficial and detrimental effects, contributing to secondary brain damage but also facilitating neurorepair [48]. To what extent cytokines, they can be defined as diagnostic factors reflecting brain injury or as predictors of long term outcome needs to be further substantiated. In clinical studies some groups have reported a correlation between cytokine production in either the cerebrospinal fluid or intraparenchymal tissue with initial brain damage, mortality, or poor outcome scores [47, 49, 50]. However, the validity of cytokines as biomarkers is not broadly accepted. This article discusses the evidence from both clinical and basic research explor-

ing the validity of such immune molecules as potential prognostic markers to classify and predict the outcome following TBI.

5. Conclusion

The kynurenine pathway of tryptophan metabolism displays a Janus face as its derived metabolites can be neurotoxic or neuroprotective depending of which cell type is producing them. This is a surprising fact but the different link of these two products to receptor of glutamate justify the bivalent function in the brain [7]. This type of molecules would represent a relevant therapeutic strategy for limiting damages in asphyctic newborns, subjects with brain trauma or stroke and other degenerative brain injuries. However, the clinical use of this inhibitor did not receive sufficiently guaranties to be proposed in conditions where the quinolinic acid is increases.

At this stage, the determination of cytokines expression profile and kynurenine pathway metabolites in brain damage requires sophisticated, time consuming and often expensive quantitative methods but in the future the automation is likely to provide more information for the treatment of asphyctic newborns and of subjects with TBI, preventing the long term disability and cognitive impairment and open new way for the prevention and treatment of these two severe pathologies.

6. Acknowledgement

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7. Authorship Contributions

Olivia Galipo'. conceived the study, Luisa Scucces, performed, analysed and associated data, Maria Cristina Morganti Kossmann, performed cytokines analysis and revising it critically for important intellectual content. Salvatore Musumeci, participated in coordination, analysis and in drafting the manuscript.

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