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The Leukocyte Count, Immature Granulocyte Count and Immediate Outcome in Head Injury Patients

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1. Introduction

Proliferation and differentiation of hematopoietic stem cells into mature white blood cells (WBC) in the bone marrow, followed by release into the circulation of mature WBC, is an extremely regulated process (Metcalf, 2008). Differentiation and maturation of the hematopoietic cells into granulocytes, monocytes, lymphocytes, megakaryocytes and erythroid cells is influenced by soluble factors including growth factors and cytokines with the bone marrow stroma, and are mediated to a certain extent through an interaction of adhesion molecules. The synchronized production of leukocytes in bone marrow is crucial for innate and adaptive immunity**.** Leukocytes encompass several subtypes including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and play a vital role in innate and adaptive immunity against invading microorganisms. They are also involved in the pathogenesis of various acute and chronic diseases. The circulating numbers of leukocytes can be influenced by stress, infection, or inflammation.

Mature neutrophils are ephemeral and localize rapidly to inflammatory sites where they deliver microbicidal activity. It takes about 14 days until it reaches the blood, of which the last 6-7 days are spent in maturation and storage pool. In less than a day after it arrives in the blood vessel, the neutrophil emigrates from the circulation in a random manner and enters the tissue. If not utilized in an inflammatory response, the neutrophils leave the body within a few days via secretions in bronchi, saliva, gastrointestinal tract and urine, or are destroyed by the reticuloendothelial system. The kinetics of eosinophils are similar to the neutrophils, they are stored in the bone marrow for several days after going through the different maturational stages. The half-life in the blood is approximately 18 hours before entering the tissues. Basophils have a life span similar to eosinophil; the maturation time in the marrow is about 7 days. Basophils circulate in the blood and are not normally found in the tissue. Monocytes share the same committed progenitor cell as neutrophils; they undergo maturation for a period of about 50-60 hours before being released in to the blood. Once monocytes enter the blood they leave randomly with a half-time of 8.4 hours. After monocytes leave the blood, they spend several months, or longer as tissue macrophages. (McPherson & Pincus, 2006).

2. Leukocyte count and immature granulocyte count as prognostic marker for traumatic brain injury

Traumatic Brain Injury persists to be a major health problem, and a recurrent cause of death and severe disability among a primarily young population. Worldwide, traumatic brain injury (TBI) is the single largest cause of death and disability following injury. Most TBI's are due to roadside accidents. According to WHO, by the year 2020, head trauma will be third largest killer in the developing world. The statistics from India are even more alarming. Studies show, on an average one person dies every six minutes, 70% of these being directly attributable to head and spinal trauma. The annual social costs of road accidents are estimated 3% of India's Gross Domestic Product (GDP). The accident rate of 35 per 1000 vehicles in India is also amongst the highest in the world (Ahmed et al., 2009). Traumatic brain injury (TBI) is also a major cause of disability, with survivors acquiring long-term cognitive, motor, behavioral or speech-language disabilities (Rutland-Brown, 2003, as cited in Namas et al., 2009). The various forms of traumatic injury therefore represent a pandemic disease that affects every nation in the world without regard for economic development, racial or religious predominance, or political ideology; this disease is acute in onset and often results in chronic, debilitating health problems affecting far beyond the individual victims (Kauvar & Wade, 2005, as cited in Namas et al., 2009).

Trauma acts as a trigger of a complex cascade of posttraumatic events that can be divided into a hemodynamic, metabolic, neuro-endocrine and immune responses leading to a multifocal pathophysiologic process (DeLong & Born, 2004, as cited in Namas et al., 2009). Inflammation is a a well-coordinated communication network operating at an intermediate time scale between neural and longer term endocrine processes which is necessary for the removal or reduction of challenges to the organism and subsequent restoration of homeostasis. (Vodovotz et al., 2008, as cited in Namas et al., 2009). Inflammation is necessary for the removal or reduction of challenges to the organism and subsequent restoration of homeostasis. (Nathan, 2002, as cited in Namas et al., 2009).

Although the inflammatory response is crucial in clearing invading organisms and offending agents and promoting tissue repair, these same responses carried out under a set of extreme conditions can also compromise healthy tissue and further exacerbate inflammation (Nathan, 2002, Jarrar, 1999, as cited in Namas et al., 2009). Thus, early identification of reliable prognostic factors for severely head-injured patients is of significance to both the practicing neurosurgeon and the clinical investigator. The ability to predict likely outcome in acutely admitted hospital patients can be beneficial in several ways. Risk assessment on the basis of laboratory investigations is also commonly used, but is usually applied in specific disease situations, and generally gives subjective assessments of risk. Several prognostic factors, such as age group, gender, pupillary reactivity, Glasgow Coma Scale (GCS) on admission, serum glucose level, total white blood cell counts, platelet counts, coagulation profile, computerised tomography (CT) scan, have been authenticated in various studies to predict outcome in adult traumatic brain injury patients.

A number of other variables have also been suggested to be important for determining the prognosis of patients with severe head injury. These include multimodality evoked potentials, electroencephalography, cerebral perfusion pressure, blood flow velocity on transcranial doppler, jugular venous oxygen saturation, brain tissue oxygenation, and specific serum biochemical markers such as creatine-kinase isoenzyme BB, neuronspecific enolase, and S-100B protein (Dings et al., 1996; Moulton et al., 1994; Nordby and Urdal, 1985;

Raabe et al., 1999; Raabe and Seifert, 2000; Sheinberg et al., 1992 as cited in Rovlias & Kotsou, 2004). Also several studies have shown that hyperglycemia and leukocytosis are associated with a worse outcome, particularly during focal ischemia or hypoxia, which are frequently found in patients with severe head injury (De Salles et al., 1987; Graham et al.,1989; Zhuang et al., 1993 as cited in Rovlias & Kotsou, 2004). Although nonreactive pupils, Sub Arachnoid Hemorrhage, acute subdural and intracerebral haematoma hold clinical significance there are routine laboratory investigations which emerge to be associated with mortality risk in hospital patients.

The complete blood count and leukocyte differential count are among the most frequently requested clinical laboratory tests. Leukocytosis, an increase in the number of circulating white blood cells, was first described by Virchow and Andral in the mid 19th century (Lawerence et al., 2007) is a common phenomenon in head injuries. High leukocyte count though nonspecific has been used more specifically as a prognostic indicator in myocardial infarction and as a predictor of plasma urinary oestrogen levels in women undergoing gonadotrophic treatment for infertility (Hughes, 1963, Cruichshank, 1970, 1972). Early trends in WBC alert the physician about the possibility of sepsis and allow prompt therapeutic response. Leukocytosis above a certain level could serve as a marker for bacterial infection despite the known physiologic leukocytosis following splenectomy (Toutouzas et al., 2002). Leukocytosis is also associated with a worse outcome, particularly during focal ischemia or hypoxia, which are frequently found in patients with severe head injury (Rovlias & Kotsou, 2004).

Leukocyte (WBC) count is considered a biomarker of inflammatory processes that actively contribute to vascular injury and atherosclerosis (Mehta et al., 1998, Alexander, 1994, as cited in Ruggiero et al., 2007). Whether elevated WBC count directly contributes to cardiovascular disease and mortality (Coller, 2005, as cited in Ruggiero et al., 2007) or is merely a marker of negative cardiovascular risk profile remains controversial (Loimaala et al., 2006, Smith et al., 2003 as cited in Ruggiero et al., 2007).

Injury educe a response from all cells of the immune system in which cytokines and other metabolic products of activated leukocytes can act either beneficially to provide for enhanced host resistance or deleteriously to depress the function of remote organs and causes systemic inflammation.

A nonspecific systemic inflammatory response occurs after both ischemic and hemorrhagic stroke, either as part of the process of brain damage or in response to complications such as deep venous thrombosis.

Inflammation alters normal leukocyte production by promoting granulopoiesis over lymphopoiesis, a response that supports the reactive neutrophilia following infection. Leukocytosis in trauma is due to neutrophilia, caused by neutrophil margination, and not due to increased marrow production or release of immature cells or bands. The phenomenon is short-lived, lasting only minutes to hours (Abramson & Beckz, 2000 as cited in Santucci et al., 2008). It is hypothesized that, patients with significant injury should have a higher degree of leukocytosis compared to patients with minor injuries (Santucci et al., 2008).

Traumatic brain injury is associated with elevated serum levels of catecholamines (Clifton et al., 1981, Hortangl et al., 1980, Rosner et al., 1984 as cited in Gürkanlar et al., 2009). Catecholamines are responsible for the release of neutrophil stores while corticosteroids cause a decrease in the egress of neutrophils from the circulation. Catecholamines increase the leukocyte count by release of the marginated cells into the circulating pool. Corticosteroids increase the neutrophil count by releasing the cells from the storage pool in the bone marrow into the blood and by preventing egress from the circulation into these

tissues (Boggs, 1967 as cited in Gürkanlar et al., 2009). Brain swelling occurring after head trauma is probably an inflammatory response due to tracerebral cytokine production and increased leukocyte adhesion as a result of a direct effect on vascular permeability and leucocyte activation (Dietrich et al., 2004, Fee et al., 2003, Gourin & Shackford, 1997, Juurlink, 2000, Lenzlinger, 2001 as cited in Gurkanlar et al., 2009). Another theory of leukocytosis after trauma can be explained as follows: In post traumatic injury, the cell body of the microglia becomes hypertrophic with long, branched and crennellated processes during the first 60 minutes post injury, as the blood brain barrier (BBB) opens at the time of the trauma and approaches closure at about 60 minutes post injury (Bednar et al., 1997 as cited in Gürkanlar et al., 2009). Microglia cells express class I and class II MHC antigens and these antigens could be presented to lymphocytes in the regional lymph nodes and trigger the activation of circulating lymphocytes in the central nervous system (Capps, 1896, Kakarieka, 1997, Neil-Dwyer & Cruickshank 1974, Rovlias & Kotsou, 2001 as cited in Gürkanlar et al., 2009). Microglia cells play a predominant role in the induction and maintenance of the immune response following head trauma (Czigner et al., 2007 as cited in Gürkanlar et al., 2009). An alternative mechanism by which leucocytes can be associated with cerebral damage is the traumatic rupture of microvessels followed by physical occlusion. The leucocytes are less deformable than the erythrocytes, and a greater pressure gradient is therefore required to force them through the capillaries with small diameter. Under conditions of reduced perfusion pressure, the capillaries may behave like a sieve and trap the leucocytes to increase the WBC count. After the entrapment, the leucocytes form a common area of contact with the endothelium and may not be dislodged even after the perfusion pressure returns to normal (Hallenbeck, 1986, Janoff, 1965, Suval, 1987, Yamakawa, 1987 as cited in Gürkanlar et al., 2009). The mechanical occlusion of the capillaries may become more evident as a result of the release of a number of cytotoxic chemicals that leads to increased leucocyte endothelial interactions (Harlan & Winn, 2007 as cited in Gürkanlar et al., 2009).

The presence of immature granulocytes (IG) in the peripheral blood may indicate bacterial sepsis, inflammation, trauma, cancer, steroid therapy or myeloproliferative diseases (Ansari-Lari et al., 2003, Briggs, 2003, 2009, Iddles, 2007.) They are also present in the later stages of pregnancy. In these cases, there is often an increased neutrophil count. Nevertheless neutrophils morphological abnormalities and automated left shift flags are notoriously unreliable as specific diagnostic features.

The measurement of the immature cells of the myeloid series, specifically "band" cells, is considered clinically useful for the diagnosis of infections, especially neonatal sepsis. Rodwell et al., 1988, Seebach et al., 1997 as cited in Buttarello & Plebani, 2008) Even though a morphologic definition of these cells exists, it is not universally accepted. (Cornbleet & Novak, 1995, as cited in Buttarello & Plebani, 2008). Immature granulocytes, normally absent from peripheral blood, are increased also in other conditions such as tissue necrosis, acute transplant rejection, surgical and orthopedic trauma. In these cases, the increase in immature granulocytes is accompanied by an increase in neutrophils, which are freed from the marginal pool and bone marrow. In some subjects, especially elderly people, neonates, and myelosuppressed patients, the increase in neutrophils may be absent, and in other conditions, such as sepsis, there can even be neutropenia. In these situations, the increase in IGs (>2%), even if isolated, can be useful for identifying an acute infection, even when not suspected (Briggs et al., 2003 as cited in Buttarello & Plebani, 2008).

Microscopic immature granulocytes counts has limits of imprecision and lack clinical sensitivity because these components are usually found in low concentrations (<10%).

Published studies agree that IG counts have a high specificity for infectious conditions (from 83% to 97%) but are accompanied by low sensitivity (between 35% and 40%) (Briggs et al., 2003, Ansari-Lari et al., 2003, as cited in Buttarello & Plebani, 2008). This low sensitivity means that this count is not indicated as a screening test for infection, even though a significant association exists between elevated IG counts and positive blood cultures (Buttarello & Plebani, 2008). The presence of low numbers of immature granulocytes is more reliably detected on automated hematology analyzers than using manual microscopy. Automated blood cell counters have undergone a formidable technological evolution owing to the introduction of new physical principles for cellular analysis and the progressive evolution of software, resulting in high number of cells being counted (Briggs et al., 2003, as cited in Briggs, 2009). This is because of the high number of cells counted and an increase of IG (>2%) can be useful in identifying infection even when not suspected (Briggs et al., 2003, as cited in Briggs, 2009).

2.1 Aim

Our aim for this study was to correlate on admission leukocyte and immature granulocyte count (IG) with the severity of head injury (according to the Glasgow coma score), computed tomography findings and pupillary reaction in trauma patients with isolated head injuries. We also intended to determine the factors influencing the immediate clinical outcome (dead or alive) in isolated head injury patients. The acute-phase response due to trauma is characterized by a leukocytosis upon admission. Therefore, an increase in the white blood cell (WBC) count might serve as an additional diagnostic and prognostic indicator in head injury. Our goal was to demonstrate that a reliable prediction of outcome based on the admission day leukocyte and immature granulocyte count is of great clinical relevance. We aimed to develop a prognostic model with readily available traditional laboratory parameters for the selection of those trauma patients who are likely to progress towards an adverse outcome, in turn ensuring their optimum management.

2.2 Materials and method

For the purpose of this study retrospective analysis of case files of patients admitted with, non penetrating head injury (mild, moderate and severe) at a level I trauma centre for duration of two months (June - July 2008) was performed. Patients with brain death, penetrating injury, infection and possible diseases that may alter the white blood cell count (myocardial infarction, cerebral vascular accident, surgical procedures etc) were excluded from the study.

Two ml of venous blood was collected in a disposable EDTA tubes, on the same day as clinical assessment or, for patients admitted to the hospital, as soon after assessment as possible, for the estimation of basic hemogram parameters and immature granulocyte count. The WBC and IG count was measured using a fully automated hematology analyzer, Sysmex XE 2100 (Sysmex, Kobe, Japan).

The results of all the routinely done, laboratory investigations were documented in the institution computerised patient record system (CPRS). The patients clinical and laboratory details were extracted from the CPRS and patient files, for the purpose of this study.

The XE-2100TM is a haematology analyser that, utilises the technology of fluorescence flow cytometry to quantitate the standard five part , immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet

count. A hemolytic reagent causes disruption of mature WBC membranes, leaving bare nuclei, while immature myeloid cells with low cell membrane lipid content remain intact. A surfactant increases membrane permeability allowing a poly-methylene dye with high affinity for nucleic acid to enter the cells. When excited by a 633-nm laser beam, the stained cells emit fluorescence proportional to their content of nucleic acid. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise but precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained. Immature granulocytes are recognized by their increased fluorescence emission compared with segmented neutrophils because they contain more RNA and DNA. The immature information (IMI) channel of the XE-2100 counts human progenitor cells (HPC). The reagents specifically affect the lipid components of the cell membranes; the membranes of mature cells, with a higher content of lipid are lysed while immature cells retain their membranes. In normal samples no intact cells are seen in the IMI area. The HPC has been shown to be an important parameter in the prediction of the apheresis yields of CD34+ cells in peripheral blood in patients undergoing progenitor cell mobilisation. It has been demonstrated that the use of peripheral blood HPC counts gives a more precise measurement of early cells than visual blast cell counts and allows a more quantitative assessment of the release of progenitor cells into the blood (Briggs et al., 1999).

The Sysmex XE 2100 automated analyzer can count immature granulocyte while performing the differential leukocyte (WBC) count, with notably lower imprecision [Coefficient Variance (CV) near 7%]. On comparison with microscopic examination or flow cytometry using Monoclonal antibody (MoAb) methods high accuracy was observed for the Sysmex XE 2100 automated analyzer (r between 0.78 and 0.96). (Briggs et al., 2003, Field et al., 2006 as cited in Buttarello & Plebani, 2008).

The corresponding computed tomography (CT) scan findings and pupillary reaction were extracted from the case files and analyzed. For purposes of analysis, pupillary reaction was noted as unilaterally present, bilaterally present or bilaterally absent reaction. The CT scan findings were recorded as subdural hemorrhage (SDH), extradural hemorrhage (EDH), intracerebral hemorrhage (ICH) and contusion and subarachnoid hemorrhage (SAH). The severity of head injury was graded according to the GCS as mild (GCS 14-15), moderate (GCS 8-13) and severe (GCS 3-7).

The WBC and IG counts were correlated with severity of head injury, pupillary reaction and CT scan findings (SDH, EDH, ICH and SAH). Death during the hospital stay was considered as the study immediate outcome and WBC count, IG count, pupillary reaction and severity of head injury were considered as its potential determinants.

Data was recorded on a predesigned proforma and managed on an excel spread sheet. Categorical variables such as pupillary reaction, severity of head injury (mild, moderate, severe), CT findings (SDH, SAH, EDH, ICH and contusion) and immediate outcome (dead/ alive) were summarized as frequency (%). Quantitative variables (WBC and IG counts) were summarized as mean±S.D (standard deviation) for normally distributed and median (Inter quartile range) for non-normally distributed variables Students's t-Test was used to compare mean values between two groups, while Wilcoxon rank sum test was used to compare median values between two groups. For the overall comparison of mean values between more than two groups, One Way Analysis Of Variance (ANOVA) followed by Bonferroni's correction in post-hoc analysis was applied.

To find out the statistical correlation of various clinical factors with the immediate outcome (dead/ alive), firstly, chi square test was used to measure the statistical association of these factors in the binary form with the outcome, followed by a bivariate logistic regression to compute unadjusted odds ratio (95% confidence interval) of each of the separate factors with the outcome. Lastly all the factors were considered simultaneously in the stepwise multivariate logistic regression analysis with probability to enter as 0.05 and the probability to remove as 0.1. STATA 10.0 statistical software (STATA corporation, Texas, US) was used for data analysis. In this study p value < 0.05 is considered as statistically significant.

2.3 Results

A total of eighty patients were included in the study. The mean age was 33.5±13.9 years; there were 70 (87.5%) males. The head injury was mild (GCS 14-15) in 17(21.3%) patients, moderate (GCS 8-13) in 21(26.2%) patients and severe (GCS 3-7) in 42 patients (52.5%). The overall admission day mean± S.D. leukocyte count and median (IQR) immature granulocyte counts were 14,062 ± 5383 cells/cumm and 0.07 (0-1.54) cells/cumm respectively. Mortality rate of 28.8% (23) was observed in the study group during the course of their hospital stay. The mean WBC count was associated with the severity of head injury according to the GCS scores, pupillary reaction and CT scan findings and the results were tabulated (table 1). The head injury patients with low GCS scores (3-7) had higher mean WBC counts compared to moderate and mild head injury groups ($p<0.001$). Head injured patients with bilaterally absent pupillary reaction had higher mean WBC counts compared to unilaterally present and bilaterally present pupillary reaction groups ($p<0.001$). However, the mean WBC count between the unilaterally present and bilaterally present pupillary reaction groups was not significant ($p<1.00$). Statistically significant association was not observed between the mean WBC count and CT scan findings. The results of the comparison between the median IG count and the other variables are shown in table 2. The median IG count in the, CT scan findings, SAH group was significantly lower than groups with other CT scan findings the non SAH group ($p=0.04$).

To dichotomize the variables, cut-offs were derived for the WBC and the IG counts. The mean WBC count among the survivors and non survivors was 12,096 ± 3842 cells/cumm and 18,934±1172 cells/cumm respectively. Similarly, the median range IG count among the survivors and non survivors was 0.07 (0-0.26) cells/cumm and 0.13 (0-1.54) cells/cumm respectively. The mean value of WBC and IG count among survivors was taken as the cut off and variables were entered in to bivariate analysis to derive the significant factors.The mean value of WBC and IG count among survivors was taken as the cut off and variables were entered in to bivariate analysis to determine the significant independent role of these investigations in head injury patients. The results of bivariate and multivariate logistic regression are shown in table 3. The non survivors had high WBC counts ($\geq$12,096 cells/cumm, $p<0.0001$), severe head injury (GCS 3-7, $p<0.001$) and higher abnormal pupillary reaction (unilaterally present and bilaterally absent pupillary reaction, $p<0.01$) compared to the survivors. Multivariate logistic regression analysis to determine the correlation of each individual factor with mortality revealed only high WBC count [OR (95% CI): 4.9 (0.8-29.5)] and severe head injury (GCS 3-7) [OR (95% CI): 4.4 (0.9-21.2)] to be independent significant predictors of mortality. Bilaterally absent pupillary responses was not found to be statistically significant in predicting mortality, thus it should not always be associated with a hopeless outcome.

Variables	Frequency (n)	WBC (Mean ± S.D.)	Statistical Significance	Post Hoc Analysis p value
Head injury (HI)				
Severe HI	42	17495.2 ± 4687.4	F = 34.1; p = 0.0001	Severe vs. Moderate : 0.001
Moderate HI	21	11114.2 ± 3557.7		Severe vs. Mild : 0.001
Mild HI	17	9223.5 ± 1933.7		
Pupillary reaction				
Bilaterally (B/L) absent	12	19533.3 ± 5884.8	F = 8.72; p = 0.0004	B/L absent vs. U/L absent : 0.009
Unilaterally (U/L) absent	11	13227.2 ± 6103.9		U/L absent vs. B/L present : 1.000
Bilaterally (B/L) present	57	13071.9 ± 4458.2		B/L absent vs. B/L present : 0.000
CT scan finding				
Extra Dural Hemorrhage (EDH)	14	11985.7 ± 4250.2	F = 1.46; p = 0.23	___
Sub Dural Hemorrhage (SDH)	19	15878.9 ± 6878.1		
Sub Arachnoid Hemorrhage (SAH)	11	14727.2 ± 5828.0		
Intra Cerebral Hemorrhage (ICH) and contusion	33	14063.6 ± 4582.7		

Table 1. Comparison of mean WBC counts in various Clinical parameters in Head Injury patients: Results of one way analysis of variance (ANOVA) and Bonferroni correction

Variables	Frequency (n)	IG median (range)	Statistical Significance	Post Hoc Analysis p value
Head injury(HI)				
Severe HI	42	0.85 (0-1.5)	$\chi^2 = 3.48$; p = 0.17	-
Moderate HI	21	0.05 (0-0.5)		
Mild HI	17	0.07 (0-0.3)		
Pupillary reaction				
Bilaterally (B/L) absent	12	0.05 (0-1.3)	$\chi^2 = 0.4$; p = 0.78	-
Unilaterally (U/L) absent	11	0.09 (0-0.3)		
Bilaterally (B/L) present	57	0.07 (0-1.5)		
CT scan finding				
Extra Dural Hemorrhage (EDH)	14	0.08 (0-0.1)	$\chi^2 = 9.68$; p = 0.04	SAH vs. EDH- p<0.05
Sub Dural Hemorrhage (SDH)	19	0.08 (0-1.5)		SAH vs. SDH - p=0.05
Sub Arachnoid Hemorrhage (SAH)	11	0.04 (0-0.2)		SAH vs. ICH - p<0.05
Intra Cerebral Hemorrhage (ICH) and contusion	33	0.08 (0-1.3)		

Table 2. Median (Inter quartile range) Immature Granulocytes (IG) counts in head injury patients: Results of Kruskal-Wallis Test and overall comparison using one way ANOVA & Bonferroni correction

2.4 Discussion

Few authors have studied the association between WBC count, outcome and head injury. Keskil et al illustrated in a study of 153 head trauma patients that WBC count exceeding 20 x 10^6/l was associated with poor clinical grade on admission and high mortality compared to those patients with normal or slightly above normal WBC counts (Keskil et al.,1994). Rovlias & Kotsou did a prospective analysis of 125 patients of severe head injury to study the prognostic significance of WBC counts in these patients. Patients with severe head injury had significantly higher white blood cell counts than did those with moderate or minor injury ($p < 0.001$). Among the patients with severe head injury, a significant relationship was found between WBC counts and Glasgow Coma Scale score, pupillary reaction, and presence of subarachnoid haemorrhage ($p < 0.001$). WBC counts were also found to be an independent significant predictor of outcome on multivariate analysis (Rovlias & Kotsou, 2001). Our results were concordant to that demonstrated by Rovlias & Kotsou. (Rovlias & Kotsou, 2001). Our results were concordant to that demonstrated by Rovlias & Kotsou.

Akin results were also seen in the study by Kan et al, wherein 146 children with Severe TBI were evaluated in attempt to establish the prognostic factors of severe TBI. They observed that a low coma score upon admission was independently associated with poor outcome, also the presence of diabetes insipidus within 3 days post-TBI (OR: 1.9), hyperglycemia (OR: 1.2), prolonged PT ratio (OR: 2.3) and leukocytosis (OR: 1.1) were associated with poorer outcome (Khan et al., 2009). In a study by Gurkanlar et al on 59 patients of head trauma, it was shown that WBC count exceeding 17.5x10^6/l had a predictive value for poor GCS score and long hospital stay. Similarly, CT progression was significantly seen in patients with moderate and severe head injury. Other studies in the literature were done on either trauma patients overall or on specific type of trauma patients such as blunt trauma victims.

Variables	**Alive (n=57)**	**Dead (n=23)**	**χ^2 Value**	**p value**	**Unadjusted odds ratio (95% CI)**	**Adjusted odds ratio (95% CI)**
WBC count ≥12,096 cells/cumm	26 (45.6)	21(91.3)	0.001	0.0001	12.5(2.6 - 58.4)	**4.9 (0.8-29.5)**
IG count > 0.07 cells/cumm	25(43.8)	14(60.8)	1.89	0.13	2.0 (0.7 -5.3)	—
Head Injury Severe	22(38.5)	20(86.9)	15.42	0.001	10.6 (2.8-39.9)	**4.4 (0.9-21.2)**
Pupillary reaction Bilaterally and Unilaterally absent(Abnormal)	12(21.0)	11(47.8)	5.73	0.01	3.43 (1.2- 9.6)	—

WBC count Cut off = 12,096 cells/cumm; IG count Cut off = 0.07 cells/cumm

Table 3. Association of various factors with the death as outcome in head injured patients: Results of bivariate and multivariate logistic regression analysis

Chang et al did a prospective analysis of 786 trauma victims and found ISS >15, GCS≤8 and white race to be associated with increase in white cell count. Their study included all trauma patients irrespective of the site of injury (Chang et al., 2003). WBC count as a laboratory marker has also been studied in blunt trauma patients to predict the severity of injury (Santucci et al., 2008).

Schnüriger et al conducted a study to ascertain the significance of serial white blood cell (WBC) counts in trauma patients with a suspected hollow viscus injury (HVI), on an overall study population of 5,950. A significant relationship between increasing Injury Severity Score and increasing WBC count on admission was found by linear regression and they concluded that WBC count elevation on admission is nonspecific and does not predict the presence of Hollow Viscus Injury (HVI) (Schnüriger et al., 2010).

Similarly, in the study done on 805 trauma patient, to test the diagnostic use of white blood cell (WBC) count in differentiating major from minor injuries. Paladino et al. concluded that WBC count was not a useful addition as a diagnostic indicator of major trauma in their study population (Paladino et al., 2010).

While the WBC had moderate discriminatory capability for serious injury, was not considered to be a reliable independent marker to rule in or out serious injury. Nevertheless, the use of WBC on presentation to the emergency department as an adjunct for making disposition decisions is recommended.

Rovlias & Kotsou observed that, WBC counts were significantly higher in those with an unfavorable outcome ($p < 0.001$) i.e. a high mean± S.D.WBC count of 18144.93±467/cumm was seen in patients with unfavourable outcome (severe disability, persistent vegetative state, dead) in contrast to a mean± S.D. WBC count of 13711.85±415.61/cumm in patients with a favourable outcome, i.e. good recovery, moderate disability (Rovlias & Kotsou, 2001). In another study, the mean WBC count was higher i.e. $21.1x10^6$/l in patients with Glasgow outcome score (GOS) of one (death) and comparatively low i.e. $12.3x10^6$/l when the GOS was five (good recovery) (Gürkanlar et al., 2009). Keskil et al showed in a prospective analysis, a low mortality rate (23%) in patients with WBC counts less than 20x106/l Compared to those with counts more than $20x10^6$/l (mortality rate 96%) (Keskil et al., 1994). The present study showed that a high WBC count (>12,096 cells/cumm) [OR (95% CI): 4.9 (0.8-29.5)] and severe head injury (GCS 3-7) [OR (95% CI): 4.4 (0.9-21.2)] to be independent significant predictors of mortality.

Rangarajan et al. in their study designed to find out the factors influencing mortality in acutely injured trauma patients receiving massive blood transfusion (MBT). They observed a total leukocyte count (TLC) ≥ 10,000 cells/cubic mm, GCS ≤ 8, the presence of coagulopathy and major vascular surgery as four independent determinants of mortality in multivariate logistic regression analysis (Rangarajan et al., 2011).

Immature granulocyte count is a recently introduced parameter in the automated hematology analyzers that provides the number of promyelocytes, myelocytes and metamyelocytes in the peripheral blood. The presence of low numbers of immature granulocytes is more reliably detected on automated hematology analyzers than using manual microscopy (Ali Ansari-Lari et al., 2003; Briggs, 2003, 2009; Iddles et al., 2007). The performance analysis of the automated hematology analyzer for this parameter has been performed earlier (Walters & Garrity, 2000). The immature granulocyte parameter measured using Sysmex-2100 is presently used only for research purposes and in the context of clinical decision making requires more prospective

trials. There are no accredited external quality assessment schemes (EQAS) available for this parameter. Nevertheless, this instrument has internal quality control material available for this parameter and has been proven to be accurate, precise and highly suitable as a screening analyzer reducing the need for manual differentials.

Bruegel et al. analyzed immature granulocytes in 156 healthy donors by using IG count and IMI channel. Men and women showed comparable values for IGs with the highest value of 0.03 $x10^9$/l for men and 0.06 $x10^9$/l for women. No age dependency for the IG counts was reported (Bruegel et al., 2004).

In the study to determine the usefulness of immature granulocyte measurement as a predictor of infection or positive blood culture. Blood samples from 102 infected and 69 non infected patients were analyzed using the Sysmex XE-2100 automated blood cell counter (Sysmex, Kobe, Japan). The percentage of immature granulocytes was found to be significantly higher ($P < .001$) in infected than in non infected patients and in patients with positive than patients with negative blood cultures ($P = .005$). Also, a percentage of immature granulocytes of > 3 was observed to be a very specific predictor of sepsis. On comparing the results of immature granulocyte measurement with total WBC count and absolute neutrophil count (ANC), receiver operating characteristic curves (ROC) showed that the percentage of immature granulocytes was a better predictor of infection than the WBC count and comparable to the ANC. They concluded that immature granulocyte measurements reflect a biologically and clinically relevant phenomenon but are not sensitive enough to be used as screening assays for prediction of infection or bacteremia. However, although infrequently encountered, a percentage of immature granulocytes of more than 3 might help expedite microbiologic laboratory evaluation of a subset of patients. (Ali Ansari-Lari et al., 2003)

In the present study, the role of admission WBC and IG count as prognostic determinants of mortality in isolated head injury patients was investigated. The study revealed that the mean WBC count was high in patients with severe (GCS 3-7) head injury and bilaterally absent pupillary reaction groups. The independent significant determinants of mortality due to head injury were high WBC count (≥12,096 cells/cumm) and severe head injury (GCS 3-7). IG count was not found to be a potential determinant of mortality in this study.

We observed high WBC counts in the non survivors compared to the survivors and the difference was statistically significant ($p<0.001$). Similar results were observed by Rovlias et al.

We found that the IG count in patients with subdural haemorrhage, extradural hemorrhage, intracerebral hemorrhage and contusion was significantly higher than those patients who had subarachnoid haemorrhage.

3. Conclusion

Leukocytosis at initial examination is associated with adverse prognosis in trauma patients. High admission WBC count (>12,096 cells/cumm) and low GCS scores (3-7) portends a worse prognosis in isolated head trauma patients. Percentage of immature granulocytes correlates with CT findings ($p=0.04$) of Head injury patients, but its association with severity of injury and mortality is clinically insignificant. More prospective studies would be

required to evaluate the role of IG count as a marker of head injury in a larger study population, also to assess whether immature granulocyte measurements could be combined with other markers to create an algorithm with better diagnostic sensitivity or specificity.

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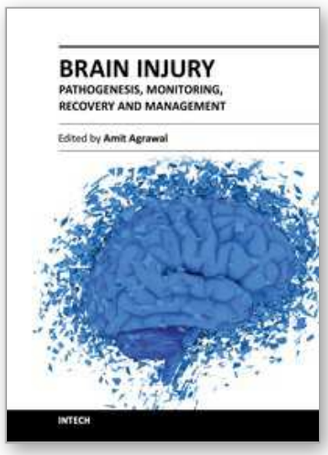

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