original paper

Haematologica 1994; 79:19-24

ANEMIA IN SURGICAL INTENSIVE CARE PATIENTS

Margaretha Viljoen, Isabella Hermiena Coetzee, Louis Johannes Roux, Jan Philip Pretorius

Department of Physiology and Department of Surgery, Faculty of Medicine, University of Pretoria, South Africa

ABSTRACT

Background. **The cause of the anemia of patients in surgical intensive care units (SICU) is not completely clear but is likely to be multifactorial. This study investigated a possible role for immune activation in the anemia of SICU patients.**

Methods. **Neopterin plasma levels, as a measure of T-cell-macrophage-axis activation, RBC-counts, Hb, Hct, MCV, MCH, MCHC, RDW, HDW, red cell morphology and iron status were determined in a group of 47 SICU patients.**

Results. **The study confirms the presence of a moderate anemia (Hb = 10.38±13 g/dL) in SICU patients. Abnormal red blood cell morphology was observed in 82% of all patients over at least part of their ICU-stay. Markedly enhanced T-cell-macrophage-axis activity was evidenced by a significant increase in the plasma neopterin levels of the patient group (44±79.6 nmol/L) compared to that of the control group (3.38±4.9 nmol/L). Iron metabolism was found to be disturbed.**

Conclusions. **The red cell distribution width, the morphological results, the enhanced macrophage activation state, as well as the results of the iron status, point towards a contribution of an immune-associated functional iron deficiency to the anemia of SICU patients.**

Key words: anemia, iron deficiency, surgery, neopterin

Many factors are known to influence the red cell status of patients in surgical intensive care units (SICU). Such factors include acute and chronic blood loss, excessive blood sampling, hemodilution, nutritional status, hydration state, transfusions and a host of other phenomena. Despite the wide spectrum of variation in the red cell status of individual patients, it would appear that SICU patients in general suffer from a moderate red blood cell (RBC) deficit. The cause of this mild anemia is not completely clear but it is likely to be multifactorial.

Surgical ICU patients in general are subject to tissue injury. This injury, irrespective of whether the cause be trauma, surgery, sepsis or even multiple organ failure, would invariably be accompanied by a degree of inflammation. Such an inflammatory process, with its diverse local and systemic physiological effects, should in theory be able to cause a decline in the RBC-numbers of SICU patients. The actual cause of the anemia would in such a case correspond to that of the so-called *hematological distress syndrome* as described in patients with chronic infections and inflammatory disorders.[1]

The relatively mild anemia associated with such disorders is said, at least partially, to be induced by immune activation – especially the activation of lymphocytes and macrophages in response to cellular injury. This chronic disease-associated anemia is characterized by a) increased monocyte/macrophage activity, b) a decrease in serum iron and an increase in storage iron – resulting from the defence strategy of activated monocytes/macrophages, c) shortened red cell survival, possibly due to increased phagocytosis, and d) a decrease in the compensatory increase of the hemopoïetic response – due to cytokine-induced bone marrow suppression.[1,2]

Neopterin, a pteridine compound containing

Correspondence: Dr. Margaretha Viljoen, Department of Physiology, Faculty of Medicine, University of Pretoria, PO Box 2034, Pretoria 0001, South Africa.

Acknowledgements: we wish to thank the typist Mrs D. Jacobs as well as Ms M. van der Walt and staff of the preclinical library.

Received on September 21, 1993; accepted December 6, 1993.

Table 1. A comparison of the highest neopterin level in each patient as well as the corresponding venipuncture red cell parameters to those of the control group.

group		neopterin (nmol/L)	RBC (x 10^{12}/L)	Hb g/dL	Hct L/L	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW	HDW
	X	3.38	4.74	15.27	40.0	89.4	29.2	34.4	12.93	2.61
controls	SD	4.9	1.0	1.91	3.0	3.9	1.2	1.3	0.67	0.37
	n	24	24	24	24	24	24	13	13	13
	X	44.62	3.52	10.38	31.04	87.8	29.6	33.3	15.6	2.93
patients	SD	79.6	0.43	1.31	3.5	6.8	2.4	2.6	2.6	0.41
	n	47	47	47	47	47	43	43	41	42
test		separate T	separate T	separate T	pooled T	pooled T	pooled T	pooled T	MW	MW
significance	(p)	0.0009*	0.0001*	0.0001*	0.0001	0.2777	0.4037	0.0589	0.0001*	0.0210*

MW: Mann-Whitney rank sum

a 2-amino, 4-oxo, pyrazine-pyrimidine (pterin) ring with a three-carbon side chain on carbon 6, is synthesized from guanosine triphosphate in macrophages and monocytes. The major stimulus for neopterin production is γ-interferon, released from activated T-cells.[3] A stimulatory role is however also implicated for α-interferon by the enhanced neopterin levels seen in patients on α-interferon therapy.[4] A correlation between immune activation, as represented by neopterin levels and the disturbed iron metabolism and anemia of chronic disease has recently been shown.[5]

The aims of this pilot study were to ascertain whether:

a) a statistically significant reduction in red cell numbers does indeed exist in SICU patients;
b) a statistically significant decline in Rbc-count/status occurs over the ICU-stay;
c) a correlation exists between the red cell concentration and the activation state of the T-cell-macrophage/monocyte-axis;
d) any morphological red cell abnormalities exist which may render these cells more susceptible to phagocytosis or may give an indication of the cause of the red cell deficit.

Methods

All patients admitted to the surgical intensive care unit over a six-month period were included in the study. The patient population (n=47) could be categorized into trauma, elective surgery, emergency surgery and sepsis/multiple organ failure groups. Control subjects consisted of hospital personnel (n=24). Blood specimens were collected into EDTA tubes at 09:00 hrs on a daily basis for the first two weeks of hospitalization and thereafter at a three times per week schedule. Total blood counts were determined by Coulter counter, neopterin by an Immuno-Biological Laboratories RIA-kit method. Serum iron was determined by HPLC, ferritin by radio-immunoassay and transferrin by nephelometry.

Results

The mean plasma neopterin levels, as well as the mean for a number of red cell parameters, i.e. RBC, Hb, Hct, MCV, MCH, MCHC, red cell distribution width (RDW) and hemoglobin distribution width (HDW), for the total patient group were compared to that of the control group. The mean values for the patient group were calculated from the highest neopterin level of each patient and the corresponding venipuncture red blood cell parameter values. Neopterin levels were significantly higher and RBC, Hb and Hct values were significantly lower in the patient than in the control group. RDW and HDW were significantly higher in the patient than in the control group (Table 1). The matched T-test was applied to test for a change in the red cell status of the patients over their ICU-stay. The mean of the first (ICU-admission) day values was compared to that for the

Table 2. A comparison between first and last day values of five red cell parameters for the total patient group.

		admission	last day	matched T
RBC ($\times 10^{12}$/L)	X	3.53	3.56	0.7847
	SD	(0.66)	(0.46)	
Hb (g/dL)	X	10.63	10.56	0.8254
	SD	(2.03)	(1.59)	
Hct (L/L)	X	31.42	31.87	0.6561
	SD	(6.16)	(3.62)	
MCV (fL)	X	87.92	89.32	0.0885
	SD	(6.23)	(5.25)	
MCH (pg)	X	29.35	29.37	0.9628
	SD	(2.30)	(1.58)	

last day values. Despite neopterin peaks around the 5th to 6th day of ICU-stay, no significant differences were evidenced between first and last day neopterin levels. Neither could significant differences be shown between first and last day values for the red cell parameters (Table 2).

In an attempt to ascertain whether the absence of differences could be ascribed to the diversity of the ICU population, patients were subsequently divided into groups. Three different subdivisions were made:

a) a subdivision into two groups, depending on whether infectious/inflammatory complications were present (complicated group) or not (uncomplicated group). There were no significant differences seen between first day Rbc-parameter values of the complicated and that of the uncomplicated group. Neither were there any between the last day red cell values of the groups. Both first day (p=0.005) and last day (p=0.0026) neopterin levels differed markedly between the two groups (Mann-Whitney test). No significant differences could be found when first day levels were compared to last day levels within a subset of either the complicated or the uncomplicated group. In other words the last day red cell status of the patients was fairly similar to that of the first day.
b) a subdivision of the patients into two groups i.e. survivors and non-survivors. No significant differences were seen between the first day values of the two groups. Significant differences were however seen in the last day values between the survivors and the non-survivors for RBC-counts (p=0.0260), Hb-concentrations (p=0.0206) and neopterin levels (p=0.0444). No significant differences were seen when first day values were compared to last day values of the same group for either the survivor or the non-survivor groups. This despite variations over the SICU-stay;
c) a subdivision of the patients into smaller groups based on diagnostic criteria, i.e. trauma, elective surgery, emergency surgery and sepsis/multiple organ failure (MOF). When first day values from these groups were compared (Mann-Whitney), significant differences were often found between groups. Such intergroup differences were also seen between groups for last day values. Only comparisons where significant differences were seen are shown in Table 3. No significant differences were however present in the comparison between first and last day values within groups for any of the diagnostic groups.

Neopterin levels, determined as a measure of T-cell-macrophage-axis activation were correlated to the red cell status in two ways:

a) an interpatient correlation where the highest neopterin level in each patient was compared to the corresponding day's red cell values. The results, as seen in Table 4, showed no correlation between neopterin and any of the red cell values, either for the patient group or for the control group. The comparison was repeated on last day values. Again no significant correlation was evidenced;
b) an intrapatient correlation where the consecutive daily neopterin levels of each specific patient were compared to the corresponding venipuncture's red cell values. The number of patients in whom this intrapatient correlation could be performed was limited to those patients who stayed long enough in SICU for a statistically significant number of daily determinations. Sixteen patients complied with the requirement. In six of these patients i.e. 37.5%, a

Table 3. Differences between groups for first day (1) and last day (2) values.

Parameter	*Day*	*Group*	*Group*	*Mann-Whitney*
		Trauma	Sepsis/MOF	
	1	11.6 ± 11.6	75.3 ± 123.9	0.0362
	2	25.4 ± 45.5	100.7 ± 200.9	0.0068
		Trauma	Emergency general surgery	
Neopterin (nmol/L)	1	11.6 ± 11.6	32.6 ± 20.1	0.0163
	2	25.4 ± 45.5	43.9 ± 25.1	0.0455
		Emergency general surgery	Elective general surgery	
	1	32.6 ± 20.1	13.3 ± 8.7	0.0393
		Trauma	Elective general surgery	
	2	25.4 ± 45.5	33.6 ± 49.1	0.0295
		Trauma	Elective vascular surgery	
	2	10.9 ± 1.2	9.3 ± 0.3	0.0438
Hb (g/dL)		Elective vascular surgery	Elective general surgery	
	2	9.3 ± 0.35	11.1 ± 1.6	0.0031
		Elective vascular surgery	Elective general surgery	
	2	28.0 ± 0.0	33.4 ± 5.0	0.0327
Hct (L/L)		Sepsis/MOF	Emergency general surgery	
	1	89.6 ± 6.0	79.8 ± 7.6	0.0285
	2	90.4 ± 5.0	81.1 ± 7.6	0.0192
MCV (f/L)		Trauma	Emergency general surgery	
	2	88.7± 4.2	81.1 ± 7.6	0.0507
		Trauma	Emergency general surgery	
	2	90.4± 5.0	81.1 ± 7.6	0.0192
		Trauma	Emergency general surgery	
	1	30.3 ± 1.4	26.1 ± 3.6	0.0335
MCH (pg)		Elective surgery	Emergency general surgery	
	1	30.5± 0.7	26.1 ± 3.6	0.0495
		Trauma	Sepsis/MOF	
	1	34.5 ± 1.2	32.9 ± 1.6	0.0465
MCHC (g/dL)		Trauma	Elective general surgery	
	1	34.5 ± 1.2	32.7 ± 1.8	0.0390

significant correlation was found between RBC-counts and neopterin levels. In three of them the correlation was positive (r=0.75773, p=0.0043; r=0.73573, p=0.0239; r=0.91532, p=0.0035), and in three negative (r=–0.63204, p=0.0370; r=–0.68105, p=0.0052; r=–0.60147, p=0.0500). Within six patients, ie 37.5% of the population, a significant correlation could be seen between the raised RDW and the raised neopterin levels of blood from consecutive days (r=0.78355, p=0.0043; r=0.61798,

Table 4. Correlations between neopterin and red cell parameter values.

group		correlation between neopterin and RBC (x 10^{12}/L)	Hb g/dL	Hct L/L	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW	HDW
	Pearson								
controls	r =	0.06038	0.17808	-0.17418	-0.01862	-0.27797	0.15721	-0.25669	0.19581
	p =	0.7793	0.4051	0.4157	0.9312	0.1885	0.4632	0.3967	0.5214
	n =	24	24	24	24	24	13	13	13
	Pearson								
	r =	0.00549	-0.00545	-0.01072	0.00227	-0.03109	-0.06280	-0.028084	0.25985
patients	p =	0.9708	0.9710	0.9430	0.9879	0.8431	0.6891	0.0753	0.0965
	n =	47	47	47	47	43	43	41	42

No significant correlations.

p=0.0427; r=0.72325, p=0.0426; r=0.62326, p=0.0405; r=0.68100, p=0.0052; r=0.87965, p=0.0001).

Microscopic examination of peripheral blood smears showed a fair degree of red cell morphological abnormality. Our own results were compared to those done by the hospital laboratory and found to correlate. Eighty-two percent (82%) of patients demonstrated anisocytosis to an abnormal degree – over at least some period of their SICU stay. This corresponds with the finding of a higher RDW. Various other red cell characteristics were observed in a varying percentage of patients, i.e. codocytes (target cells) in 64%, polychromasia in 60%, hypochromasia in 56%, elliptocytes (ovalocytes) in 49%, spherocytes in 31%, macrocytes in 22%, schizocytes in 20%, erythroblasts in 18%, microcytes in 16%, basophillic stippling in 11%, poikilocytes in 9%, rouleaux in 9%, pencil cells in 9%, stomatocytes in 7%, dacrocytes in 7%, burr cells in 2% and megaloblasts in 2% of patients.

The results of the neopterin levels and the morphological results prompted an investigation into the iron status of the patients. Plasma iron, transferrin and ferritin were subsequently determined on the stored plasma of a random sample from the patient group (n=22). Last day blood specimen were used for the analyses. Results are shown in Table 5.

Table 5. Iron status of a random sample from the patient group (n = 22).

	plasma iron (µmol/L)	*transferrin (g/L)*	*ferritin (µg/L)*	*transferrin saturation*
surgical group (n = 11)	9.8 ±13.2	2.2 ±0.3	284.5 ±248.9	20.7% ±8.1
trauma group (n = 7)	9.3 ±2.9	2.28 ±0.35	258 ±39	18.8% ±7.6
sepsis/MOF (n = 4)	21.3 ±7.9	2.03 ±0.4	3013 ±2885	53.3% ±34.3
pooled stored controls (n = 10)	23.0	2.77	26	37.1%
laboratory	10-30	2.52-4.29	30-233	25-50%

Discussion

The study confirmed the presence of a mild, but significant anemia in surgical intensive care patients. The severity of the anemia is to a degree masked by the ICU policy of blood transfusions as soon as the hematocrit drops below 30. This practice may further partially underlie the lack of a significant decline in the red cell numbers over the ICU-stay period.

Marked differences are however seen between admission day values and last day values when red cell parameters of different subgroups are compared. Such differences include a significantly greater decline in the hemoglobin concentration of a) the non-survivors relative to the survivors, b) the elective vascular surgery group vs the trauma group, and c) the elective vascular surgical group vs the elective general surgery group.

Red cell morphological abnormalities were commonly found in the majority of patients, and the finding of anisocytosis in 82% of patients over at least part of their ICU stay was confirmed by the wider than normal red cell distribution width of the patient group. Several factors, such as the high incidence of anisocytosis, polychromasia, hypochromasia, elliptocytes and ovalocytes pointed towards a possible iron deficiency.

The presence of significantly enhanced T-cell-macrophage-axis activation state was borne out by the magnitude of the neopterin increase. Significantly greater macrophage activity was seen a) in SICU patients where inflammatory/infectious complications were evident than in patients without visible signs of such complications, b) in the non-surviving than in the survivor group, c) in the sepsis/MOF group than in the trauma group, d) in the emergency general surgery group than in the trauma group, and e) in the elective general surgery group than in the trauma group. A direct correlation between red cell status and T-cell-macrophage-axis activity, as implicated by neopterin production, could however not be shown. In view of the relatively acute nature of the immune stimulation in SICU patients - relative to the stabilized immune activation of chronic inflammatory and infectious diseases - this was to be expected.

The results of the iron status investigation on a random sample from the patient group indicate a functional iron deficiency in the majority of patients, where available iron is sequestered from the circulation into storage. Interferon-γ-stimulated macrophages, during the immune response and during immunosurveillance, are said to involve a functional iron depletion.[6] It is thus possible that the iron shift observed in this study might, in view of the high neopterin levels recorded, represent part of the macrophage defence strategy against infection.

In conclusion, this study indicates that the anemia of surgical ICU patients may be exacerbated by a functional iron deficiency secondary to enhanced immune activation.

References

1. Erslev AJ. Anemia of chronic disorders. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA, eds. Hematology, 4th ed. New York: McGraw-Hill, 1990.
2. Means RT, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease. Blood 1992; 80:1639-47.
3. Schoeden G, Troppmair J, Adolph G, Huber C, Niederwieser A. Interferon-γ enhances biosynthesis of pterin in peripheral blood mononuclear cells by induction of GTP-cyclohydrolase activity. Interferon Res 1986; 6:697-703.
4. Caenazzo A, Pietrogrande F, Sgarabotto D, et al. Serum neopterin levels in hematological malignancies. Hematologica 1993; 78:225-9.
5. Wachter H, Artner-Dworzak E, Fuchs D, et al. Association between neopterin and iron metabolism in cancer patients. Tumordiagn U Ther 1990; 11:229-32.
6. Weinberg ED. Iron depletion: a defence against intra-cellular infection and neoplasia. Life Sci (England) 1992; 50:1289-97.