Anemia in ST-Elevation Myocardial Infarction Patients with Markers of Inadequate Bone Marrow Response

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ABSTRACT: **Background:** Anemia confers an adverse prognosis in patients with ST-elevation myocardial infarction (STEMI). Several mechanisms have been implicated in the etiology of anemia in this setting, including inflammation, blood loss, and the presence of comorbidities such as renal failure.

Objectives: To evaluate the adequacy of bone marrow response as potentially reflected by elevation in blood and reticulocyte counts.

Methods: Consecutive men with STEMI who underwent primary percutaneous intervention within 6 hours of symptom onset and who presented to our catheterization laboratory during a 36 month period were included in the study. The cohort was divided into quartiles according to hemoglobin concentration, and differences in clinical and laboratory characteristics between the groups were evaluated.

Results: A total of 258 men with STEMI were recruited, 22% of whom suffered from anemia according to the World Health Organization classification (hemoglobin < 13 g/dl). Men in the lowest quartile of hemoglobin concentration presented with significantly lower white blood cell and platelet counts (9.6 ± 2.9 vs. 12.6 ± 3.6 x103/µl, $P < 0.001$) and (231 ± 79 vs. 263 ± 8 x103/µl, $P < 0.01$), respectively, despite higher inflammatory biomarkers (C-reactive protein and fibrinogen) compared with patients in the upper hemoglobin concentration quartile. Reticulocyte production index was not significantly higher in anemic patients, with a value of 1.8, 1.4, 1.5 and 1.6 in the ascending hemoglobin quartiles, respectively ($P = 0.292$).

Conclusions: Anemic men with STEMI have relatively lower leukocyte and platelet counts as well as a reduced reticulocyte count despite higher inflammatory biomarkers. These findings might suggest inadequate bone marrow response.

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KEY WORDS: ST-elevation myocardial infarction (STEMI), anemia, bone marrow, white blood cells (WBC), inflammation

Anemia confers an adverse prognosis in patients with ischemic heart disease [1,2], particularly in those with acute coronary syndromes (ACS) [3]. Several mechanisms have been implicated in the etiology of anemia in this population, including anemia of inflammation [4], blood loss due to treatment modalities and repeated blood tests [5,6], and the presence of other comorbidities including chronic kidney disease (CKD) and diabetes mellitus (DM) [7].

The high prevalence of anemia and the adverse prognosis of anemic patients with ST-elevation myocardial infarction (STEMI) is not fully explained by the suggested mechanisms as mentioned above. Blood loss does not explain the high prevalence of baseline anemia and ongoing anemia months after the event or the adverse outcome it confers [2,3,6]. Similarly, inflammation of chronic disease caused by STEMI does not account for the high prevalence of anemia at baseline in this population [2-4]. Furthermore, anemia in STEMI patients is a negative prognostic factor independent of other comorbidities such as DM or CKD [7]. Finally, correction of anemia by either blood transfusion [8-10] or erythropoietin analogues [11] has not been shown to improve prognosis in patients with ACS.

We hypothesized that inadequate bone marrow function may be an additional cause of anemia in patients presenting with STEMI. In order to study this possibility we analyzed the results of complete blood counts and reticulocyte count in a group of men with STEMI who presented to our center within 6 hours from the onset of chest pain to assess whether, in addition to anemia, these patients also demonstrate reduced platelet and leukocyte count or a maladaptive reticulocyte production index (RPI) as possible markers of bone marrow dysfunction

PATIENTS AND METHODS

The dataset included in this study was collected as part of the Tel Aviv Prospective Angiographic Survey (TAPAS). The TAPAS is a prospective single-center registry that enrolls all patients undergoing cardiac catheterization at the Tel Aviv Sourasky Medical Center [4,12]. For the present study, we selected all men with STEMI who were treated within 6 hours from symptom onset and who presented to our catheterization laboratory during a 36 month period. We studied men in order to avoid confounders of chronic anemia in women

due to menstruation or low iron storage pool. The 6 hour time limit for symptoms was set in order to avoid anemia secondary to bleeding and repeated blood tests and anemia secondary to chronic inflammation. All patients underwent routine primary percutaneous coronary intervention (PCI). Written informed consent was obtained from all subjects, and the study was approved by the institutional ethics committee.

DEFINITION OF CARDIOVASCULAR RISK FACTORS

- Diabetes mellitus: defined as the patient having been informed of this diagnosis by a physician prior to admission, patients receiving hypoglycemic treatments (dietary, oral anti-diabetic agents, or insulin), or those who presented to the catheterization laboratory with the diabetic hallmark serum hemoglobin (Hb) A1c level ≥ 6.5%
- Hypertension: defined as known elevation of blood pressure on at least two separate occasions according to the medical history, or the use of antihypertensive medications in a patient with known controlled hypertension
- Dyslipidemia: determined by the medical history, the use of lipid-lowering medications, or fasting serum low density lipoprotein (LDL) levels > 160 mg/dl
- Smoking status: ascertained by the medical history
- STEMI: defined according to current guidelines [13]
- Anemia: defined according to the World Health Organization (WHO) classification (hemoglobin concentration < 13 g/dl in men) [14].

LABORATORY TESTS

Arterial blood was obtained from all participants via their arterial access puncture site as part of the coronary angiography procedure. Blood counts were determined using the Coulter STKS™ electronic cell analyzer (Beckman Coulter, Nyon, Switzerland). Quantitative fibrinogen analysis was performed by the method of Clauss and using a Sysmex 6000™ autoanalyzer (Sysmex Corporation, Hyaga, Japan). Blood chemistry was measured using the Advia system. Reticulocyte production index (RPI) was calculated according to standard procedures [15]. Transferrin, ferritin and iron levels were also measured by the Advia system.

STATISTICAL ANALYSIS

Categorical variables were compared using the chi-square test and continuous variables by *t*-test (presented as means with standard deviations, SD) or by the Kruskal-Wallis/ Mann-Whitney test (medians with interquartile range, IQR). Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test and Q-Q plots. Correlations were conducted using Spearman's or Pearson's correlation tests, respectively. Multivariate logistic regression was used to evaluate whether lower hemoglobin and reticulocyte levels are associated with inadequate bone marrow function by adjusting for clinical factors including age, gender, glomerular filtration rate (GFR), and risk factors. A two-tailed $P < 0.05$ was considered statistically significant. All analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, USA).

RESULTS

The study population comprised 258 consecutive male patients. Their mean age ± SD was 61 ± 13 years. The mean hemoglobin concentrations were 13.9 ± 1.3 g/dl (median 13.9 g/dl, IQR 13.2–14.8 g/dl); 22% percent suffered from anemia according to the WHO classification (< 13 g/dl).

We divided this group into quartiles according to hemoglobin (Hb) concentration. Clinical characteristics according to Hb quartiles are reported in Table 1. Anemic patients were older and had a more prevalent hypertensive disease than non-anemic subjects. Intake of oral hypoglycemic medications was more common in the anemic group (data not shown) while no difference in other medications that might cause bleeding such as antiplatelet or anticoagulant drugs was noted between anemic and non-anemic patients.

Anemic patients had decreased renal function and lower serum protein concentrations (both total and albumin) compared with non-anemic patients, suggesting that these patients may have had more profound systemic morbidity or a more pronounced inflammatory state. Patients with lower hemo-

Table 1. Clinical characteristics of the study population according to hemoglobin quartiles

	Hemoglobin quartile (g/dl)				
	Quartile I (12.2 ± 0.9)	Quartile 2 (13.6 ± 0.2)	Quartile 3 (14.3 ± 0.2)	Quartile 4 (15.5 ± 0.5)	
	n=63	n=69	n=58	n=68	*P* value
Age, years (range)	67 ± 14 (24–95)	59 ± 11 (36–84)	59 ± 10 (35–83)	56 ± 12 (31–88)	‹ 0.001
Males	63 (100%)	69 (100%)	58 (100%)	68 (100%)	NA
Current smokers	45 (71%)	27 (39%)	21 (36%)	34 (50%)	0.09
Hypertension	41 (65%)	21 (30%)	20 (35%)	26 (38%)	‹ 0.001
Dyslipidemia	37 (59%)	38 (55%)	32 (55%)	38 (56%)	0.97
Ischemic heart disease	26 (41%)	31 (45%)	19 (33%)	27 (40%)	0.57
Prior stroke	5 (8%)	2 (3%)	3 (5%)	0 (0%)	0.11
Peripheral vascular disease	7 (11%)	8 (12%)	4 (7%)	8 (12%)	0.79
Diabetes mellitus	18 (29%)	12 (17%)	11(19%)	10 (15%)	0.22

Clinical characteristics are shown according to hemoglobin quartiles. First, second, third and fourth hemoglobin quartiles included 63, 69, 58 and 68 subjects respectively. Hemoglobin concentrations were 12.2 ± 0.9, 13.6 ± 0.2, 14.3 ± 0.2 and 15.5 ± 0.5 in quartiles 1 to 4, respectively

Table 2. Complete blood cell count according to hemoglobin quartiles

	Hemoglobin quartile (g/dl)				
	Quartile I (12.2 ± 0.9)	Quartile 2 (13.6 ± 0.2)	Quartile 3 (14.3 ± 0.2)	Quartile 4 (15.5 ± 0.5)	
	n=63	n=69	n=58	n=68	*P* value
Hemoglobin (g/L)	12.2 ± 0.9	13.6 ± 0.2	14.3 ± 0.2	15.4 ± 0.5	‹ 0.001
MCV (fl)	87 ± 6.5	89 ± 4.2	89 ± 4	89 ± 5	0.24
MCH (pg/cell)	29 ± 2.5	30 ± 2	30 ± 1.5	31 ± 1.8	0.17
RDW (%)	14 ± 1.5	15.6 ± 1	13.5 ± 0.9	13.5 ± 1	0.28
WBC (x10³/µl)	9.6 ± 2.9	10.8 ± 3.4	11 ± 3.5	12.6 ± 3.6	‹ 0.001
NEU (%)	74 ± 11	75 ± 10	74 ± 11	75 ± 9	0.811
LYM (%)	17 ± 9	17.5 ± 8	19 ± 8.5	18 ± 8	0.54
Monocytes (%)	6.7 ± 2.5	5.6 ± 2.2	5.8 ± 2.1	5.4 ± 1.9	0.006
Eosinophils (%)	1.2 ± 1	1.2 ± 1	1.5 ± 1.3	1.3 ± 1	0.38
Basophils (%)	0.45 ± 0.27	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.2	0.54
PLT (1000/µl)	231 ± 79	230 ± 49	259 ± 61	263 ± 82	0.01
MPV (fl)	9.3 ± 1.2	9.5 ± 1	9.2 ± 1	9 ± 1	0.39

Leukocyte and platelet counts are presented in addition to blood cells indices

MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, RDW = red cell distribution width, WBC = white blood cells, NEU = neutrophils, LYM = lymphocytes, PLT = platelets, MPV = mean platelet volume

Table 3. Laboratory results of possible causes of anemia according to hemoglobin quartiles

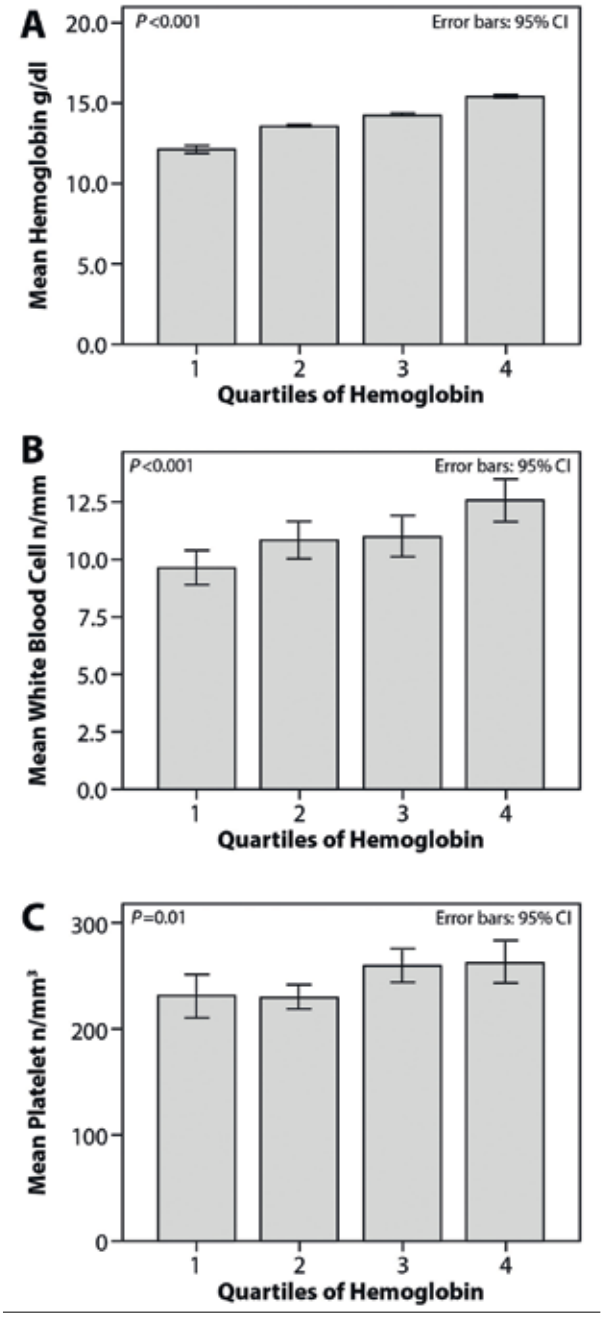
	Quartile I (12.2 ± 0.9)	Quartile 2 (13.6 ± 0.2)	Quartile 3 (14.3 ± 0.2)	Quartile 4 (15.5 ± 0.5)	
	n=63	n=69	n=58	n=68	*P* value
Reticulocyte (%)*	1.6 ± 0.6	1.3 ± 0.5	1.4 ± 0.5	1.6 ± 0.4	0.89
RPI*	1.8	1.4	1.5	1.6	0.29
Iron (µg/dl)	41 ± 21	68 ± 26	62 ± 18	67 ± 20	0.01
Transferrin saturation	13 ± 5	20 ± 8	20 ± 6	19 ± 7	0.02
Transferrin (mg/dl)	237 ± 70	236 ± 24	231 ± 30	251 ± 44	0.49
Ferritin (ng/ml)	157 ± 228	153 ± 107	129 ± 80	170 ± 107	0.06
GFR (ml/min)	71 ± 29	86 ± 27	86 ± 25	91 ± 25	‹ 0.001

Some relevant tests for evaluating anemia including: ferritin, iron, transferrin and % transferrin saturation, reticulocytes and their production index

*Reticulocyte data were available for only 12%, 17%, 19% and 21% of subjects in Hb quartiles 1, 2, 3 and 4 respectively

RPI = reticulocyte production test, GFR = glomerular filtration rate

Figure 1. Blood counts according to hemoglobin quartiles. The white blood cell and platelet counts are shown according to hemoglobin quartiles. First, second, third and fourth hemoglobin quartiles included 63, 69, 58 and 68 subjects respectively



globin levels had higher inflammatory biomarkers: highly sensitive C-reactive protein (hs-CRP) and fibrinogen (data not shown).

Table 2 presents the results of the complete blood count. White blood cell (WBC) count was 9.6, 10.8, 11.0, 12.6 x10³/µl in ascending Hb quartiles respectively. The platelet count was 231, 230, 259, 263 x10³/µl in ascending Hb quartiles, respectively. The data are presented graphically in Figure 1. The correlations of Hb concentrations to both WBC ($r = 0.29$, $P < 0.001$) and platelet counts ($r = 0.17$, $P = 0.006$) were significant.

Table 3 demonstrates the relevant tests for evaluating anemia. Patients in the lowest quartile demonstrate signifi-

cantly lower transferin saturation as well as lower iron levels compared to higher Hb quartiles (P = 0.02 and P = 0.01, respectively). However, there were no signficiant differences in the percent of reticulocytes or in RPI between the quartiles (P = 0.89 and P = 0.29, respectively).

After adjusting for age, renal function and iron levels, patients in the lower quartile had similar reticulocyte levels (P = 0.84) as well as similar renal function (P = 0.76) compared to other quartiles. In addition, the differences in WBC and platelets also became non-significant (P = 0.12 and P = 0.83, respectively).

DISCUSSION

The present study demonstrates a correlation between decreasing hemoglobin concentrations and reduced white blood cell and platelet counts in light of a mounting inflammatory state in men with acute STEMI. In addition, despite suffering from anemia, subjects in the lowest Hb quartile did not develop adequte reticuloctye response.

We selected a study population to exclude confounding variables that may contribute to anemia in acute STEMI, i.e., we excluded women because of their potential for iron depletion and, in addition, we restricted the time from symptom onset to 6 hours in order to avoid anemia secondary to bleeding or repeated phlebotomy [5,6].

A remarkable finding to support our notion on the presence of an eventual bone marrow dysfunction was the relatively low leukocyte and platelet counts without elevation of reticuloctyes in subjects in the lowest quartile of hemoglobin, who, according to two established inflammatory biomarkers (hs-CRP and fibrinogen), had more inflammation.

The direct association between the three blood counts in the presence of a significantly higher inflammatory state in the anemic patients, in contrast to the expected inverse relation usually seen in inflamation, i.e., thrombocytosis and leukocytosis, may resemble the maladaptive reaction of the elderly to infection associated with a worse prognosis, as described by Lipschitz et al. [16], reflecting to some extent malfunctioning of the bone marrow. Indeed, the differences in WBC and platelets also became non-significant (P = 0.12 and P = 0.83, respectively) after correcting for age and renal function, respectively, thus supporting this connection.

As seen in Table 3, the lower Hb group has some iron deficiency with low iron and transferin saturation that might be partially due to an increased inflammatory state. Although these findings explain to some extent the presence of anemia, a higher RPI was to be expected as a compensatory response. The RPI [Table 3] which did not significantly increase with decreasing hemoglobin quartiles and remained < 2 in the lowest Hb quartile, might be another indicator for relative bone marrow insufficiency. Our results did not change much after multivariate analysis adjusting for age, renal function and other laboratory variables, and the RPI index did not increase despite reduced hemoglobin levels.

As discussed, the currently proposed pathophysiology of anemia in patients presenting with STEMI does not fully account for the prevalence of anemia in this population, its presence at presentation and its adverse impact on prognosis [2-4]. Increased comorbidity of patients in the lower Hb quartile in the present study population, who were older and more hypertensive, may be related to a partially malfunctioning bone marrow as such comorbidites are known to be inversely related to the number and function of circulating endothelial progenitor cells [17-19], a marker for bone marrow functionality [20].

It has been shown that the bone marrow's ability to produce progenitor cells that are involved in endothelial repair and release them into the circulation is imperative for intact endothelial function in patients undergoing PCI [22] and those with stent thrombosis [22] and is associated with a lower risk of cardiovascular morbidity in the general population [23].

Lee et al. [24] showed that bone marrow response to the stress of ACS as demonstrated by release of circulating endothelial cells is a major determinant of future cardiovascular events and the only inflammatory marker independently associated with death. Impairment of endothelial progenitor cell function in the bone marrow and in peripheral blood is related to unfavorable remodelling of the left ventricle after MI [25]. This finding may explicate, at least in part, the well-known but only partially understood adverse prognosis of ACS patients who are anemic.

We postulate that anemia in acute STEMI may be a marker for inadequate bone marrow function, given our findings of a direct relationship between the three blood counts, in contrast to the predicted inverse correlation in an increasing inflammatory state.

LIMITATIONS

The main limitation of the present study was the relatively small number of patients. This limitation did not allow us to assess the impact of our findings on the outcome. We did not have laboratory results for other possible causes of anemia, such as folic acid and vitamin B12. In addition, we did not have data on outcome (future blood counts or cardiovascular outcome), or on infarct size and location.

CONCLUSIONS

Our findings of lower leukocyte and platelet counts associated with hemoglobin decline despite increasing inflammation, and lack of significant increase in reticulocyte production index are possible clues to the involvement of the bone marrow in the pathophysiology of anemia in STEMI.

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