BASELINE LEUKOCYTE COUNT AND ACUTE CORONARY SYNDROME: PREDICTOR OF ADVERSE CARDIAC EVENTS, LONG AND SHORT TERM MORTALITY AND ASSOCIATION WITH TRADITIONAL RISK FACTORS, CARDIAC BIOMARKERS AND C-REACTIVE PROTEIN

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Background: The elevated WBC count has been accepted as part of healing response following myocardial infarction as well as a predictor of adverse cardiovascular events. The study was designed to find out correlation between WBC count and coronary risk factors, cardiac biomarkers, C-reactive protein (CRP), incidence of adverse cardiac events and mortality in patients of ACS in Pakistan. **Methods:** One hundred and thirty-three patients of ACS were stratified according to WBC categories, WBC1 (<7000/mm^3), WBC2 (7100–10,000/mm^3) and WBC3 (>10,000/mm^3). The WBCs were counted on admission by Sysmex cell counter, CRP by immunoturbidimetric method, and CK-MB and Trop-I by enzyme immunoassay. Adverse cardiac events and mortality were recorded for 12 months of follow up period. **Results:** Long term mortality in patients with ACS was 6.4% in WBC1, 18.2% in WBC2 and 40.9% in WBC3 categories, while short term mortality was 2.6%, 3.0% and 18.2% in WBC1, WBC2, and WBC3 categories respectively. Relative to patients in lower 2 WBC categories, patients in the highest category were 7 times more likely to die during 30 days (HR 7.83, p=0.017) and more than 9 times during the total follow up period (HR 9.42, p<0.001). Cox regression analysis showed WBC3 a strong independent predictor of mortality (HR 6.36, p=0.016). WBC count showed a positive correlation with coronary risk factors, cardiac biomarkers and CRP. **Conclusion:** WBC count is a strong independent predictor of mortality in patients with ACS and has positive correlation with coronary risk factors, cardiac biomarkers and CRP.

Keywords: Acute coronary syndrome, WBCs, Mortality, C-reactive protein

INTRODUCTION

Inflammation plays a key role in the development of atherosclerosis (AS) and acute coronary syndromes, the most important cause of sudden cardiac death, acute myocardial infarction (AMI) and unstable angina (UA).[1] Certain inflammatory markers have been identified to be associated with increased incidence of cardiovascular complications and death in patients with ACS. Among these biomarkers some have emerged to be potentially useful in early risk assessment of patients with ACS, such as CRP, B type natriuretic peptide, troponin, interleukin 6, and white blood cell (WBC) count.[2]

Despite the potential prognostic importance of these novel biochemical markers, many of them are not routinely available. On the other hand WBC count is a simple test that is available universally and is considered to be one of the most commonly obtained tests in the emergency department. It can be applied immediately at the bed site with no investment in new infrastructure or tests. In addition, every provider is familiar with it and its interpretation in routine clinical practice.[3]

The elevated WBC count typically indicates infection and inflammation and plays a role in the atherogenesis, development of atherosclerotic plaque rupture and thrombosis. Recent studies have shown that WBCs destabilize coronary artery plaque at the onset of ACS, and the elevated WBC count is considered to be an independent predictor of long term cardiac mortality especially following AMI and related events both in healthy individuals free of coronary heart disease (CHD) at base line and in patients with ACS.[4]

The relationship between elevated WBC and CHD is strong, consistent, temporal, dose dependent and biologically plausible. The acute MI triggers a systemic response to a necrotic insult characterized by leukocytosis and acute phase protein synthesis.[5] The elevated WBC count plays a central role in the reparative process that take place to replace the necrotic tissue for collagen. Epidemiological studies have found a strong association of basal WBC count with future cardiovascular disease and adverse outcome.[6]

The present study is designed to determine predictive role of WBC count on admission for mortality in patients of ACS; where lack of resources keeps the access of so many to the best diagnostic methods, WBC count may become an additional parameter for the preliminary approach of patients with ACS.

PATIENTS AND METHODS

This cohort study was performed in a population of 133 consecutive patients (mean age 60.9±11.9 years, 63.2% male) admitted with a diagnosis of ACS and on 69 (mean age 59.7±14.3 years, 66.7% male) controls.

Within the patients of ACS, 37 were diagnosed as patients of STEMI, 38 of non-STEMI and 59 as patients of UA.

All patients were evaluated by taking detailed history and physical examination. The variables included in the study were age, sex, hypertension (HTN), diabetes mellitus (DM), smoking, hypercholesterolemia, family history of IHD, ECG, cardiac biomarkers (Trop I, CK-MB), CRP and white blood cell count.

The inclusion criteria for the patients of ACS were those of American College of Cardiology and European Society of Cardiology.[7]

The criteria for STEMI were as follows: an increase in the levels of myocardial necrosis (troponin I >1 ng/ml); new ST elevation from the J point in two or more contiguous leads with an elevation of at least 0.2 mV in leads V1, V2 and V3 or at least 0.1 mV in the remaining leads during the first 24 hours following the onset of the symptoms. Patients were also included if a new ST-segment elevation in the presenting electrocardiogram was associated with a recent episode of chest pain but in whom it was not possible to obtain analysis of myocardial necrosis.

The criteria for definition of NSTEMI were: increased levels of markers of myocardial necrosis (as for STEMI) along with the presence of either symptoms of ischemia or alterations of ST-segment (except persistent ST-segment) elevation.

Criteria for UA were: typical chest pain and ECG modifications, such as ST depression or T wave inversion in ≥2 leads, absence of new or presumed new echocardiographic alterations and cardiac wall motion abnormalities.

Patients with history of infection or systemic inflammation during the last 15 days, or with hepatic, renal or haematologic disease at admission, and those who did not sign the informed consent proforma were excluded from the study.

Venous blood under aseptic condition was withdrawn from all the patients at the time of admission and was analysed for CRP, CK-MB, Trop I and WBC count.

The plasmatic CRP levels were estimated with particle enhanced immunoturbidimetric method based on antigen-antibody reaction using Hitachi 911 from Roche Diagnostic Germany. The CK-MB and Trop I were determined by Microparticle Enzyme Immuno Assay (MEIA) based on 'sandwich' principle using AxSYM system from Abbott Laboratories USA.

WBCs were counted by automated cell counter Sysmex SE9000, Sysmex Corporation, Kobe, Japan. The levels were expressed in units of $10^3/mm^3$. The counts were assigned into three categories, WBC1 (4×10^3–$7\times10^3/mm^3$), WBC2 (7.1×10^3–$10\times10^3/mm^3$) and WBC3 ($>10\times10^3/mm^3$) cut off value was according to the previous studies.

Follow up was done for a period of one year through outpatient department or through telephonic contact. The specified primary end point was death from cardiac cause and the secondary end point was non fatal MI/UA.

The statistical analyses were performed using SPSS version 10.0. The qualitative variables were expressed as percentage and compared using the χ^2 test. Continuous variables were described as Mean±SD. The multivariate Cox regression analysis was performed to identify independent predictors of death. Difference was considered statistically significant at a value of $p<0.05$.

RESULTS

The baseline clinical and demographic characteristics of patients with ACS and controls are shown in Table1. The most prevalent coronary risk factor in patients of ACS was systolic HTN (Odds ratio 3.77, 95% CI, 1.66–8.56, $p=0.001$), followed by hypercholesterolemia, smoking, and diabetes mellitus, who showed a significant relation with ACS as compared to controls. Similarly, the percentage of cardiac biomarkers (CK-MB, Trop I) and CRP was significantly raised ($p<0.001$) in patients with ACS versus controls. Although the p values for percentage of males, family history of IHD and DM, and patients above 60 years were less than 0.05; however, the statistical significance was unclear because the 95% confidence interval (CI) of the Odds ratio crossed over 1.0.

Table-2 depicts baseline characteristics according to the category of WBC count on admission in patients with ACS. The patients in the highest category were more often smoker, hypertensive (systolic and diastolic) and were more often diabetic, hypercholesterolemic with a positive family history of IHD, and showed a significant difference ($p<0.05$) than patients in the other categories. The patients of the WBC3 category (WBC count >10,000/mm^3) had increased proportion of short and long term mortality and an increased concentration of CK-MB, Troponin I and CRP levels compared to patients of other two categories. In contrast, no significant difference was seen between the WBC categories when male, patients above 60 years with a positive family history of diabetes and hypertension were compared.

The association between WBC count on admission and outcome is shown in Table-3. There were 7 deaths and 5 non-fatal MI/UA during 30 days follow up period, which increased to 20 deaths and 15 non fatal AMI/UA during total follow up period of 12 months. The deaths were from the cardiac causes. Mortality in 30 days (short term) and during total

follow up period of 12 months (long term) was significantly higher with the highest WBC category relative to other two categories in patients of ACS. Relative to the patients in other two categories, the patients in the highest category were 7 times more likely to die during 30 days and more than 9 times during total follow up period of 12 months. Similarly, the patients in highest category were about 2 times more likely to have non fatal AMI/UA in 30 days and 5 times during the 12 months follow up period. The combined end point of death and non fatal AMI/UA was significantly higher in patients with highest category versus other categories, and was more than 4 times in 30 days and more than 7 times in 12 months follow up.

Table-4 indicates independent predictors of long term mortality in ACS patients. All baseline and clinical variables shown in Table-1 were entered in multivariate Cox regression analysis; after adjustment for these factors, WBC >10,000/mm^3 was found to be strongest independent predictor of long term mortality. Relative to patients with other categories, there was 22 fold increases in the risk of death in patients with highest WBC category. Other independent predictors of mortality were CRP, WBC2 category, cholesterol, diabetes mellitus, Trop I, and systolic hypertension.

In patients with ACS a positive correlation of total leukocyte count with systolic pressure (r=0.306, p<0.001), diastolic pressure (r=0.194, p=0.025), serum total cholesterol (r=0.326, p<0.001), blood glucose levels (r=0.442, p<0.001), cigarette smoking (r=-0.237, p=0.006), CK-MB (r=.188, p=0.030) and Trop I (r=0.229, p=0.008) was seen, while correlation was negative with serum HDL (r=0.067, p=0.447), LDL cholesterol (r=0.037, p=0.672) and total triglycerides (r=0.122, p= 0.161).

Table-1: Baseline clinical characteristics of patients with ACS and controls

	Patients of ACS	Controls	Odds ratio	95% CI	*p*-value
Age >60 years (%)	55.6	50.7	1.22	0.68–2.18	0.506
Male (%)	63.2	66.7	0.86	0.46–1.58	0.621
Smoking (%)	38.3	20.3	2.44	1.23–4.84	0.009
Systolic HTN (%)	33.1	11.6	3.77	1.66–8.56	0.001
Diastolic HTN (%)	27.1	10.1	3.29	1.38–7.85	0.005
DM (%)	45.1	29.0	2.01	1.08–3.75	0.026
Hypercholesterolaemia (%)	39.1	20.3	2.52	1.27–4.99	0.007
Family h/o IHD (%)	35.3	29.0	1.34	0.71–2.51	0.363
Family h/o HTN (%)	12.0	24.6	0.42	0.19–0.89	0.022
Family h/o DM (%)	17.3	18.8	0.90	0.42–1.91	0.785
CRP>5mg/L (%)	45.1	13.0	5.48	2.51–11.95	<0.001
CK-MB>9.4 ng/ml (%)	47.4	01.4	61.20	8.25–453.77	<0.001
Troponin I>1.0 ng/ml (%)	50.4	01.4	69.03	9.31–511.79	<0.001

ACS= acute coronary syndrome; CI= confidence interval; HTN= hypertension; h/o= history of; IHD= ischemic heart disease, DM= diabetes mellitus, CRP= C-reactive protein, CK-MB= MB fraction of creatine kinase. (Values are expressed in percentage)

Table-2: Baseline characteristics according to category of white blood cell count on admission in patients with ACS

	1st category 4000–7000/mm^3 n=78	2nd category 7100–10,000/mm^3 n=33	3rd category >10,000/mm^3 n=22	*p*-value
Age >60 (years)	57.7	48.5	59.1	0.630
Male (%)	60.3	66.7	68.2	0.706
Smoking (%)	30.8	36.4	68.2	0.006
Systolic HTN (%)	16.7	45.5	72.7	<0.001
Diastolic HTN (%)	15.4	36.4	54.5	<0.001
DM (%)	34.6	33.3	100.0	<0.001
Hypercholesterolaemia (%)	30.8	27.3	86.4	<0.001
Family h/o IHD (%)	24.4	39.4	68.2	0.001
Family h/o HTN (%)	07.7	18.2	18.2	0.187
Family h/o DM (%)	19.2	21.2	04.5	0.217
CRP>5 mg/L (%)	32.1	42.2	95.5	<0.001
CK-MB >9.4 ηg/ml (%)	35.9	48.5	86.4	<0.001
Troponin I>1.0 ηg/ml (%)	39.7	51.5	86.4	0.001
Mortality				
Short term mortality	02.6	03.0	18.2	0.045
Long term mortality	06.4	18.2	40.9	<0.001

ACS= acute coronary syndrome; HTN= hypertension; DM= diabetes mellitus; h/o= history of; IHD= ischemic heart disease; CRP= C-reactive protein; CK-MB= MB fraction of creatine kinase. (Values are expressed in percentage)

Table-3: Association between white blood cell count on admission and outcome in patients of ACS

	1st category 4000–7000/mm^3 n=78	2nd category 7100–10,000/mm^3 n=33	3rd category >10,000/mm^3 n=22	Hazard ratio (95% CI)	*p*-value
30 Days follow up					
Non fatal MI/UA	2 (2.6%)	2 (6.1%)	1 (4.5%)	1.98 (0.18–21.84)	0.577
Death	2 (2.6%)	1 (3.0%)	4 (18.2%)	7.83 (1.43–42.80)	0.017
Combine end point of death & non fatal MI/UA	4 (5.1%)	3 (9.1%)	5 (22.7%)	4.92 (1.32–18.32)	0.018
Total follow up (12 months)					
Non fatal MI/UA	4 (5.1%)	7 (21.2%)	4 (18.2%)	5.00 (1.24–20.13)	0.023
Death	5 (6.4%)	6 (18.2%)	9 (40.9%)	9.42 (3.14–28.27)	<0.001
Combine end point of death & non fatal MI/UA	9 (11.5%)	13 (39.4%)	13 (59.1%)	7.42 (3.16–17.43)	<0.001

CI= confidence interval; MI= myocardial infarction; UA= unstable angina. (Values are expressed in percentage)

Table-4: Independent predictors of long term mortality in patients with ACS using multivariate analysis

	Odds ratio (95% CI)	*p*-value
WBC 7100–10,000/mm^3	6.36 (1.41–28.72)	0.016
WBC >10,000/mm^3	22.45 (2.03–249.1)	0.011
CRP-0 (>5 mg/L)	14.80 (2.82–77.58)	0.001
Cholesterol (>200 mg/dl)	3.92 (0.89–17.22)	0.070
Diabetes (>140 mg/dl)	2.18 (0.42–11.30)	0.352
Troponin I (>1ng/ml)	1.76 (0.42–7.47)	0.440
Systolic hypertension (>140 mmHg)	1.06 (0.35–3.22)	0.916
Smoking	0.26 (0.053–1.23)	0.090
Family history of IHD	0.27 (0.075–0.95)	0.042

WBC=white blood cells; CRP=C-reactive protein; IHD= ischemic heart disease

DISCUSSION

Our study showed that WBC count determined on admission predicts short term and long term mortality in the early risk stratification of patients with ACS. Despite adjusting for co-variables, WBC acted as an independent predictor of short term and long-term mortality. These findings provide indirect evidence in favour of an independent role of WBC count in the pathogenesis of post-AMI complications.

The results are in consistence with Nunez *et al*[5] who had showed adverse outcome in patients with ACS with elevated baseline leukocyte count. Initially Haines *et al*[8], showed significantly raised leukocyte count at presentation in patients with AMI as compared to UA, later Cannon *et al*[9] showed high mortality in patients with AMI or high risk UA, who had leukocyte count >10,000/ml. An association between increased baseline WBC count and higher incidence of cardiac complications after AMI, was shown by Menon *et al*[10] in particular with short and long term mortality.

Acute MI triggers a systemic response to a necrotic insult characterised by leukocytosis and acute phase protein synthesis. In this setting, elevated WBC count plays a central role in reparative process that takes place to replace the necrotic tissue for collagen. In addition to be a proxy for the intensity of the peri-infarction inflammatory response, recent evidence has also shown that an elevated WBC count, measured during the acute phase of MI, to be associated with adverse outcome.[5]

We found that patients with highest category were more likely to have adverse cardiac events (non fatal AMI/UA) and death than patients with lower categories. The results are in agreement with Muller *et al*[11], and Hung *et al*[12], who stratified the patients into quartiles by leukocyte count, and showed that the patients in the high leukocyte quartile were 3.3 times more likely to die of cardiac causes than those in the lowest quartile.

A number of mechanisms explain the actions of leukocytes in the coronary heart disease as follows: the endothelial cell injury caused by proteolytic and oxidative damage, vessel plugging effecting the blood flow through the cardiac microvasculature, decrease perfusion[13], hyper-coagulable state with decrease epicardial patency and increased ischemic burden, abnormal leukocyte aggregation blocking micro-vessels similar to platelets and increased expression of monocytes tissue factors[14]. Other mechanisms include, effect on blood flow, association with atherosclerotic risk factors, electrical instability, increased thrombus formation in CHD, involvement in haematological stress syndrome, increased leukocyte adhesion in CAD and expansion of infarction.[15] The prognostic value of inflammatory markers is seen across a wide clinical spectrum of atherosclerotic diseases, from their role in plaque pathogenesis to their usefulness in quantifying the inflammatory response during AMI.[16]

Regarding extent of infarction various studies have related WBC to the variables associated with the extent of the AMI, as peak level of iso-enzyme MB of creatine kinase (CK-MB), and left ventricular ejection fraction.[9] The role of leukocytes in infarct expansion following AMI is reparative and is initiated to replace the necrotic tissue with the scar tissue; suggesting that greater the amount of necrosis the larger the leukocyte response. A relation between extent of necrosis and level of local and systemic leukocyte response is also seen.[17]

In our study, proportion of population with hypertension (systolic and diastolic), diabetes, smoking and hypercholesterolemia showed a

monotonic increase from WBC1 to WBC3 in patients with ACS indicating an indirect association of WBC with the extent of AMI. Further analysis of the data showed a positive correlation between WBC count with blood glucose levels, systolic and diastolic blood pressure, serum total cholesterol, cigarette smoking, CK-MB, Trop I and CRP, but is inversely proportional with serum triglyceride, HDL and LDL cholesterols. The results are in agreement with study that has shown a positive correlation of leukocyte count with cigarette smoking, serum total cholesterol, haematocrit, blood glucose levels and diastolic blood pressure and inversely with serum HDL cholesterol.[18]

CONCLUSIONS

WBC count is a strong independent predictor of short and long term mortality in patients with ACS and may be used as a tool for risk stratification of patients with ACS. WBC count has a positive correlation with coronary risk factors, cardiac biomarkers and CRP.

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