Association between baseline neutrophil count, clopidogrel therapy, and clinical and angiographic outcomes in patients with ST-elevation myocardial infarction receiving fibrinolytic therapy

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Aims
To investigate the association between neutrophil count, outcomes, and benefit of clopidogrel therapy in ST-elevation myocardial infarction (STEMI).

Methods and results
Baseline neutrophil count was measured in 2865 patients in CLARITY-TIMI 28, a randomized trial of clopidogrel vs. placebo in STEMI patients undergoing fibrinolysis. Angiography was performed at 2–8 days following enrolment. Analyses were adjusted for demographics, time from symptom onset, Killip class, peak CK-MB, and therapies received. A baseline neutrophil count in the highest quartile was independently associated with the risk of cardiovascular (CV) death [adj (adjusted) OR (odds ratio) 5.8, \( P < 0.001 \)] and congestive heart failure (adj OR 3.0, \( P = 0.009 \)) at 30 days. Patients with higher neutrophil counts were less likely to achieve complete ST-segment resolution (adj OR 0.76, \( P = 0.03 \)) or TIMI myocardial perfusion grade 2/3 (adj OR 0.71, \( P = 0.017 \)). Clopidogrel had a lesser effect on reducing the odds of a closed infarct-related artery, or death or MI before angiography, in patients with a neutrophil count above the median (adjusted OR 0.83, 0.61–1.13) vs. in those below the median (adjusted OR 0.46, 0.33–0.64) (\( P_{\text{interaction}} = 0.008 \)).

Conclusion
In patients with STEMI, higher baseline neutrophil count is associated with worse angiographic findings and increased CV mortality, as well as a diminished benefit of clopidogrel.

Keywords
Acute coronary syndrome • ST-elevation myocardial infarction • Fibrinolysis • White blood cell • Clopidogrel

Introduction
Inflammation has been established as an important contributor to atherothrombosis and reperfusion injury during an acute coronary syndrome (ACS). To that end, several studies have shown an association between leukocyte count and both angiographic and clinical outcomes in patients with ACS. As well, platelet activation and aggregation play a central role in initiating and propagating thrombosis, and antiplatelet therapy is a key component in the management of ACS.

Clopidogrel has been shown to improve infarct-related artery patency and improve clinical outcomes in patients receiving fibrinolytic therapy for management of ST-elevation myocardial infarction (STEMI). By blocking the P2Y12 receptor, clopidogrel inhibits platelet activation and aggregation. Moreover, as pathways between thrombosis and inflammation are interconnected, it has

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have previously been reported.8,20 In brief, CLARITY-TIMI 28 was a double-blind, randomized, multicentre clinical trial of clopidogrel vs. placebo in addition to fibrinolytic therapy in 3491 patients with STEMI who presented within 12 h of symptom onset. The choice of fibrinolytic and type of heparin were left at the discretion of the managing physician. Exclusion criteria relevant to this analysis include age <18 or ≥75 years, use of clopidogrel in the 7 days prior to randomization, contraindications to fibrinolytic drugs, planned elective angiography within 48 h or evidence of cardiogenic shock. The protocol was approved by the relevant institutional review boards, and written informed consent was obtained from all patients.

As part of the trial protocol, patients were scheduled to undergo coronary angiography 2–8 days after initiation of therapy to assess for late patency of the infarct-related artery. Angiography was permitted prior to 48 h only if clinically indicated. The decision for coronary revascularization was left to the discretion of the managing physician. Patients were followed for clinical outcomes and adverse events through to 30 days following the time of randomization.

**Outcomes**
Clinical endpoints for this analysis included cardiovascular (CV) death, recurrent myocardial infarction (MI), congestive heart failure (CHF) and recurrent ischaemia leading to urgent revascularization through to 30 days follow-up. Follow-up data at 30 days were available in 3487 of 3491 (99.9%) subjects. Endpoints were defined according to previously reported criteria.8,20 All ischaemic events were adjudicated by a Clinical Events Committee that was blinded to assigned treatment arm. Information on the development of new or worsening CHF was collected from the case report forms.

Angiographic flow data including TIMI flow grade and TIMI myocardial perfusion grade (TMPG) were assessed in a blinded manner as previously defined at the TIMI Angiographic Core Laboratory.21,22 On electrocardiography, complete ST-segment resolution was defined as ≥70% resolution after 180 min.23 All electrocardiograms were interpreted at the TIMI Electrocardiography Core Laboratory by investigators who were blinded to treatment assignment and outcomes.

**Blood sampling and analysis**
Baseline neutrophil count, total white blood cell count, monocyte count, and cardiac biomarkers of necrosis were assessed in the laboratories at the local enrolling institution and recorded on the case report form. Lymphocyte count was estimated as the total non-neutrophil and non-monocyte white blood cell count.

**Statistical analysis**
Continuous and categorical variables were compared using the χ² test for trend. The correlation between baseline neutrophil count and other variables were examined using Spearman’s correlation coefficient. Baseline neutrophil count was modelled either as a continuous variable or divided into quartiles or at the median for analysis. Logistic regression was used to model the relationship between neutrophil count and outcomes adjusting for age, sex, race, diabetes mellitus, current tobacco use, prior MI, prior aspirin use, prior statin use, time from symptom onset to receiving fibrinolytic, Killip class II–IV, initial type of heparin (low molecular weight heparin, unfractionated heparin or none), type of fibrinolytic (fibrin-specific or non-fibrin-specific lytic), infarct location (anterior or other), peak creatinine kinase-MB and randomized treatment arm. Covariates for the multivariable model were selected on the basis of either being significantly imbalanced between neutrophil quartiles or being an established predictor of CV death in patients with STEMI. Linearity assumption for continuous variables was assessed by examining the stepwise increase in risk across equal steps.

Angiographic analyses were restricted to those patients who underwent coronary angiography as per trial protocol (94%). Patients who underwent percutaneous intervention (PCI) prior to 180 min or who did not receive fibrinolytic therapy were excluded from electrocardiographic analyses. All tests were two-sided with a P-value <0.05 considered to be significant. Due to the exploratory nature of the current analysis, no adjustments were made to thresholds for significance.

**Results**

**Baseline characteristics**
Baseline neutrophil count was recorded in 2865 patients whose baseline characteristics are displayed in Table 1. Patients with an available neutrophil count did not differ significantly from the overall CLARITY-TIMI 28 trial population by age, gender, past medical history, infarct size or baseline Killip class (data not shown). The mean [± standard deviation (SD)] neutrophil count for the study population was 7.8 ± 3.5 × 10⁹/L and was measured a mean of 3.3 ± 2.1 h from symptom onset.

Baseline neutrophil count was similar regardless of age or sex. Patients with an elevated neutrophil count were significantly more likely to be active smokers. Conversely, patients with a lower neutrophil count at presentation were more likely to have been treated with prior statin or aspirin therapy.

In terms of the index presentation, there was no association between neutrophil count and infarct territory location, but patients with an elevated baseline neutrophil count were more likely to have a longer time from symptom onset, more likely to have evidence of heart failure (Killip class II–IV) at the time of presentation, more likely to receive a non-fibrin specific lytic, less likely to receive UFH, and less likely to have undergone PCI. Although patients with higher baseline neutrophil counts had higher peak CK and CK-MB levels, the degree of correlation with baseline neutrophil count was modest (r = 0.28, P < 0.001 for baseline CK-MB, r = 0.12, P < 0.001 for peak CK-MB). There existed a weak correlation between baseline neutrophil and platelet count (r = 0.15, P < 0.001).
Association of baseline neutrophil count to clinical outcomes

Baseline neutrophil count (mean ± SD) was significantly higher in patients who died from CV causes as compared with patients who survived to 30 days (9.4 ± 3.5 vs. 7.7 ± 3.5 × 10⁹/L, P < 0.001). A 1-SD elevation in baseline neutrophil count was associated with a 12% increase in the odds of CV death over 30 days [OR (odds ratio) 1.12, 95% CI 1.08–1.17, P < 0.001]. When considered as a categorical variable, a strong graded relationship was observed between baseline neutrophil quartile and the risk of CV death by 30 days, ranging from 1.4% for patients with a neutrophil count in the lowest quartile to 7.4% for those in the highest quartile (P < 0.001 for trend, Figure 1).

The association between higher baseline neutrophil count and the risk of CV death by 30 days was consistent across several important subgroups (defined on the basis of imbalances across the quartiles and prior associations with CV death), including time from symptom onset to fibrinolytic therapy, infarct size as estimated by peak CK-MB, and PCI during hospitalization (Table 2).

After adjusting for demographics, diabetes mellitus, current tobacco use, prior MI, prior medications, time from symptom onset to treatment, Killip class, infarct location, peak CK-MB,
type of lytic, type of heparin, and treatment arm, a 1-SD elevation in baseline neutrophil count was independently associated with an increased risk of CV death at 30 days (adjusted OR 1.12 per SD, 95% CI 1.06–1.19, P < 0.001). In particular, patients with a baseline neutrophil count in the top quartile had more than a five-fold increase in the odds of CV death as compared with patients with a baseline neutrophil count in the lowest quartile (adjusted OR 5.8, 95% CI 2.3–14.1, P < 0.001).

Baseline neutrophil count was also significantly higher in patients who developed CHF during the first 30 days, as compared with those who did not (8.9 ± 3.6 vs. 7.7 ± 3.5 × 10⁹/L, P < 0.001). A 1-SD elevation in baseline neutrophil count was associated with a 9% increase in the odds of CHF over 30 days (OR 1.09, 95% CI 1.04–1.14, P < 0.001). Similarly, baseline neutrophil count was associated with a step-wise increase in the odds of developing CHF, ranging from 2.2% for patients with a neutrophil count in the lowest quartile to 6.0% for patients with a neutrophil count in the highest quartile (P < 0.001 for trend, Figure 2). When modelled as a continuous variable, a 1-SD elevation in baseline neutrophil count was independently associated with a higher risk of developing CHF, even after adjusting for the aforementioned variables (adjusted OR 1.10 per SD, 95% CI 1.03–1.17, P = 0.004). Similarly, baseline neutrophil count in the highest quartile was independently associated with a three-fold increase in the risk of developing CHF as compared with patients with a neutrophil count in the lowest quartile (adjusted OR 3.0, 95% CI 1.3–6.7, P = 0.009). In contrast, baseline neutrophil count was not significantly associated with the risk of recurrent MI or recurrent ischaemia leading to urgent revascularization.

**Association of neutrophil count with electrocardiographic findings**

Patients with a higher baseline neutrophil count were significantly less likely to achieve complete ST-segment resolution at 180 min (P < 0.001 for trend, Figure 3), as compared with patients with a lower baseline neutrophil count. After adjusting for the aforementioned variables, baseline neutrophil count above the first quartile remained independently associated with lower odds of complete ST-segment resolution following fibrinolytic therapy (adjusted OR 0.76, 95% CI 0.59–0.97, P = 0.03).

**Association of baseline neutrophil count with angiographic findings**

As per the trial protocol, all patients were required to undergo coronary angiography 2–8 days following randomization to assess infarct-related artery patency. Baseline neutrophil count in the highest quartile was significantly associated with lower odds of myocardial microvascular perfusion at the time of angiography, as defined by TMPG 2/3 (adjusted OR 0.71, 95% CI 0.56–0.97, P = 0.017). After adjusting for baseline variables, baseline neutrophil count in the highest quartile was not associated with late patency of the infarct-related artery, as defined by a TIMI flow grade of 2/3 (adjusted OR 1.0, 95% CI 0.68–1.5, P = 0.99), the percent stenosis of the culprit artery, the presence of thrombus, or the extent of coronary artery disease at the time of angiography.

![Figure 1](image)

**Figure 1** Association between baseline neutrophil count quartile and the risk of cardiovascular (CV) death by 30 days follow-up.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Number</th>
<th>Odds ratio (95% confidence interval)</th>
<th>P interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>2865</td>
<td>3.0 (1.4–6.1) 3.1 (1.5–6.4) 5.6 (2.8–11.2)</td>
<td>N/A</td>
</tr>
<tr>
<td>Time from randomization to fibrinolytic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 h</td>
<td>782</td>
<td>3.6 (1.6–8.0) 3.8 (1.7–8.2) 6.6 (3.1–14.0)</td>
<td>0.71</td>
</tr>
<tr>
<td>2–4 h</td>
<td>1288</td>
<td>3.8 (1.1–13.5) 3.0 (0.8–11.2) 8.4 (2.5–28.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;4 h</td>
<td>795</td>
<td>3.3 (0.7–15.2) 3.6 (0.8–16.0) 4.3 (1.0–18.7)</td>
<td></td>
</tr>
<tr>
<td>Peak CK-MB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; median</td>
<td>1158</td>
<td>2.0 (0.7–6.0) 1.8 (0.6–5.6) 7.5 (2.8–20.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>≥ median</td>
<td>1156</td>
<td>4.9 (1.1–22.5) 5.5 (1.2–24.4) 7.0 (1.6–30.2)</td>
<td></td>
</tr>
<tr>
<td>PCI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1523</td>
<td>5.7 (1.2–26.2) 2.6 (0.5–14.5) 7.2 (1.6–33.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>No</td>
<td>1342</td>
<td>2.1 (0.9–4.8) 2.5 (1.1–5.6) 4.0 (1.9–8.7)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Association between baseline neutrophil count and the risk of cardiovascular death by 30 days within specified subgroups
Comparison of neutrophil count with other cell counts

The risk of CV death associated with an elevation in baseline neutrophil count was compared with that of other cell counts including total WBC, monocyte count, estimated lymphocyte count and the neutrophil-to-lymphocyte ratio (Table 3). The baseline monocyte count, lymphocyte count, and neutrophil-to-lymphocyte ratio were not significantly associated with either angiographic findings or clinical outcomes. Total white blood cell count was significantly associated with clinical outcomes, but not nearly as strongly as neutrophil count.

Baseline neutrophil count and benefit of treatment with clopidogrel

Overall, the CLARITY-TIMI 28 study demonstrated that treatment with clopidogrel significantly reduced by 36% (24–47%, \( P < 0.001 \)) the odds of the primary composite endpoint of TIMI flow grade 0/1, or death or MI prior to angiography, when compared with placebo. When we explored the benefit of clopidogrel by baseline neutrophil count, patients with a baseline neutrophil count greater than the median (\( \geq 7.19 \times 10^9/L \)) appeared to derive lesser benefit from treatment with clopidogrel, as compared with patients with a baseline neutrophil count below the median (adjusted \( P \) interaction = 0.008). Specifically, clopidogrel reduced the odds of the primary endpoint by 54% in patients with a baseline neutrophil count less than the median (adjusted OR 0.46, 95% CI 0.33–0.64), as compared with only a 17% odds reduction for patients with a baseline neutrophil count greater than the median (adjusted OR 0.83, 95% CI 0.61–1.13, Figure 4). Directional consistency for this finding was seen across both elements of the primary endpoint including TIMI flow grade 0/1 and death or MI prior to angiography.

Discussion

We have demonstrated that baseline neutrophil count is a strong and independent predictor of the risk of CV death and CHF in a large population of patients with STEMI receiving contemporary fibrinolytic and antithrombotic therapy. Underpinning this association may be our observations that an elevated neutrophil count is independently associated with a reduced probability of early successful reperfusion and late myocardial microvascular perfusion. Interestingly, the benefit of clopidogrel for improving infarct-related artery patency following fibrinolysis may be attenuated in patients with an elevated baseline neutrophil count.

Table 3 Comparison of the risk associated with an elevation of different cell and the risk of cardiovascular death by 30 days

<table>
<thead>
<tr>
<th>Cell counta</th>
<th>Adjusted odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quartile 2 vs. quartile 1</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>2.9 (1.1–7.6) ( P = 0.03 )</td>
</tr>
<tr>
<td>Total white blood cell count</td>
<td>0.8 (0.4–1.6) ( P = 0.45 )</td>
</tr>
<tr>
<td>Monocyte count</td>
<td>0.7 (0.4–1.4) ( P = 0.32 )</td>
</tr>
<tr>
<td>Estimated lymphocyteb count</td>
<td>0.8 (0.4–1.5) ( P = 0.43 )</td>
</tr>
<tr>
<td>Neutrophil/estimated lymphocyteb ratio</td>
<td>1.8 (0.8–3.7) ( P = 0.14 )</td>
</tr>
</tbody>
</table>

\( a \)Cell count comparisons were restricted to those patients with an available white blood cell count with differential (\( n = 2865 \)).

\( b \)Lymphocyte count is estimated based on the total non-neutrophil and non-monocyte white blood cell count.
Numerous cross-links are known to exist between the thrombotic and inflammatory pathways in the pathophysiology of ACS. A higher platelet count has been shown to be associated with an increased risk for adverse outcomes in patients with ACS.24 Both leukocytes and platelets adhere to the endothelial lining at sites of vascular injury. In addition, neutrophils migrate into the vessel wall and microvasculature in response to ischaemia and reperfusion.25–27 Interactions between leukocytes and platelets via cell surface receptors further propagate this cycle through upregulation of the CD11b/18 receptor on the leukocyte surface, leading to further propagation of pro-inflammatory cytokines from the leukocyte.33 The impaired myocardial perfusion seen in patients with an elevated baseline neutrophil count may in part be mediated directly by a leukocyte-mediated no-reflow state in the microvasculature.31 The impaired myocardial perfusion seen in patients with an elevated baseline neutrophil count may in part be mediated directly by a leukocyte-mediated no-reflow state in the microvasculature.31

In terms of reperfusion, we found that a higher baseline neutrophil count was associated with a lower probability of achieving complete ST-segment resolution. ST-segment resolution is regarded as valuable surrogate for assessing tissue level reperfusion at both the epicardial and microvascular level following fibrinolytic therapy.24,35 Consistent with our findings, Barron et al. found that baseline leukocyte count was a significant predictor of a closed infarct-related artery and reduced myocardial perfusion when angiography was performed in the early hours following fibrinolytic therapy. This observation may help to explain why we did not find an association between neutrophil count and the risk of recurrent MI, since patients who do not achieve initial vessel reperfusion are not at risk of reinfarction in the absence of viable myocardium.

We did, however, find that elevated baseline neutrophil count is an independent predictor of impaired late myocardial perfusion. Of interest, clopidogrel appeared to have a diminished benefit toward improving infarct-related artery patency in patients with a higher baseline neutrophil count. In addition, baseline neutrophil count was inversely associated with late vessel patency in patients treated with clopidogrel. These findings are consistent with the proposed mechanism of action for clopidogrel in the setting of fibrinolytic therapy, namely, that clopidogrel helps to maintain infarct-related artery patency by preventing epicardial and microvascular reocclusion rather than by increasing the probability of initial vessel reperfusion.35 Since we found that a lower baseline neutrophil count is associated with an improved likelihood of ST segment resolution and therefore early patency, clopidogrel may offer the most benefit in this population by helping to maintain a patent vessel. Alternatively, patients with a higher neutrophil count may exhibit non-platelet mediated thrombosis or have increased numbers of circulating leukocyte-platelet aggregates that are relatively resistant to antiplatelet therapy.

The limitations of the current analysis include that it is an observational post-hoc analysis. Baseline neutrophil count was not available in all patients in CLARITY-TIMI 28 as ascertainment was not mandatory; however, the baseline characteristics of those patients in this analysis did not differ from the overall trial. Although we adjusted extensively for important baseline factors that differed in patients across baseline neutrophil quartiles, we cannot exclude residual confounding. We did not perform corrections for multiple hypothesis testing as our work builds on multiple previous associations of leukocyte count, angiographic findings, and CV death and heart failure.

Although we have hypothesized on possible mechanistic explanations for our findings, it is not possible to establish causality in the current analysis. As such, further exploration will be necessary to determine if leukocytes function primarily as a marker of risk or if they contribute directly toward the pathogenesis of the observed outcomes. Future prospective studies will also be required to...
evaluate the selection of specific cutpoints for defining risk and to determine whether baseline neutrophil count may help to identify patients who will benefit most from particular treatment strategies.

In conclusion, we found a strong association between baseline neutrophil count and several clinical and angiographic outcomes in a large dataset of patients receiving fibrinolytic therapy for STEMI. In particular, an elevated baseline neutrophil count is independently associated with an increased risk of CV death and CHF, despite modern antithrombotic therapy including clopidogrel. This relationship may in part be explained by a lower probability of successful reperfusion and reduced sustained myocardial perfusion in patients with an elevated leukocyte count. These findings lend further support for therapies targeting both platelets and leukocytes in STEMI and may be useful for helping clinicians guide patient risk assessment.

Conflict of interest: M.S.S. has received honoraria from Sanofi-Aventis and Bristol-Myers Squibb.

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