Profiling of chronic myeloid leukaemia patients' platelets *ex vivo* before and after treatment with Imatinib

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Background

Chronic myeloid leukaemia (CML) is a malignancy of the hematopoietic stem cells resulting in the hyperproliferation of leukocytes. The pathogenesis of CML results from the translocation of the Philadelphia (Ph) chromosome from stem cells in the bone marrow. Treatment of CML is primarily through administration of tyrosine kinase inhibitors (TKIs). The first line of treatment for CML, especially in developing countries, remains the first generation TKI, Imatinib.

Patients with CML are frequently diagnosed with platelet abnormalities, specifically thrombocytosis. Bleeding abnormalities are reported to be due to irregular platelet function owing to the clonal proliferation of hematopoietic cells. However, the specific mechanism of platelet abnormalities in CML, whether due to disease pathogenesis or TKI treatment, remains unclear and poorly understood.

The aim of this study was therefore to determine platelet morphology, activation and the apoptotic profiles of chronic myeloid leukaemia patients *ex vivo* on platelets before and after treatment with Imatinib.

Methodology

Blood samples of 30 healthy volunteers and 6 CML patients at diagnosis and after six months of treatment with Imatinib were collected. Platelet morphology, counts, viability and activation were determined by scanning electron microscopy (SEM), transmission electron microscopy (TEM), human cluster of differentiation 41 and -62 and possible occurrence of early apoptosis was measured by means of flow cytometry via Annexin V-fluorescein isothiocyanate.

Results

Data revealed that CML patients' platelet counts were elevated upon diagnosis and levels statistically significantly decreased after 6 months of treatment with Imatinib (P-value of <0.05) (**Figure 1**). Platelet activation was statistically significantly increased after 6 months of treatment with Imatinib compared to levels at diagnosis (P-value < 0.05) (**Figure 2**) which was corroborated by SEM and TEM morphology findings (**Figure 3 and 4**). Similarly, platelet apoptosis was also increased after 6 months of treatment with Imatinib in CML patients (**Figure 5**).

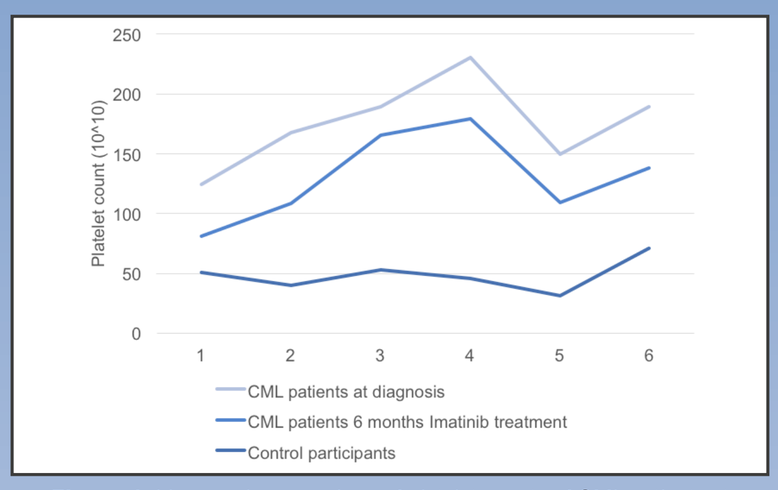

Figure 1: Line graph comparison of platelet counts of CML patients at diagnosis, after 6 months of treatment with Imatinib and control participants.

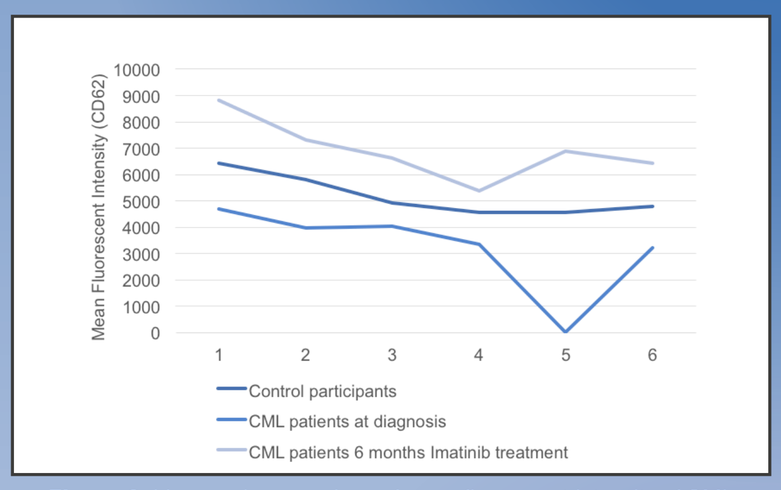

Figure 2: Line graph comparison of mean fluorescent intensity of CML patients at diagnosis, after 6 months of treatment with Imatinib and control participants with the CD62 marker.

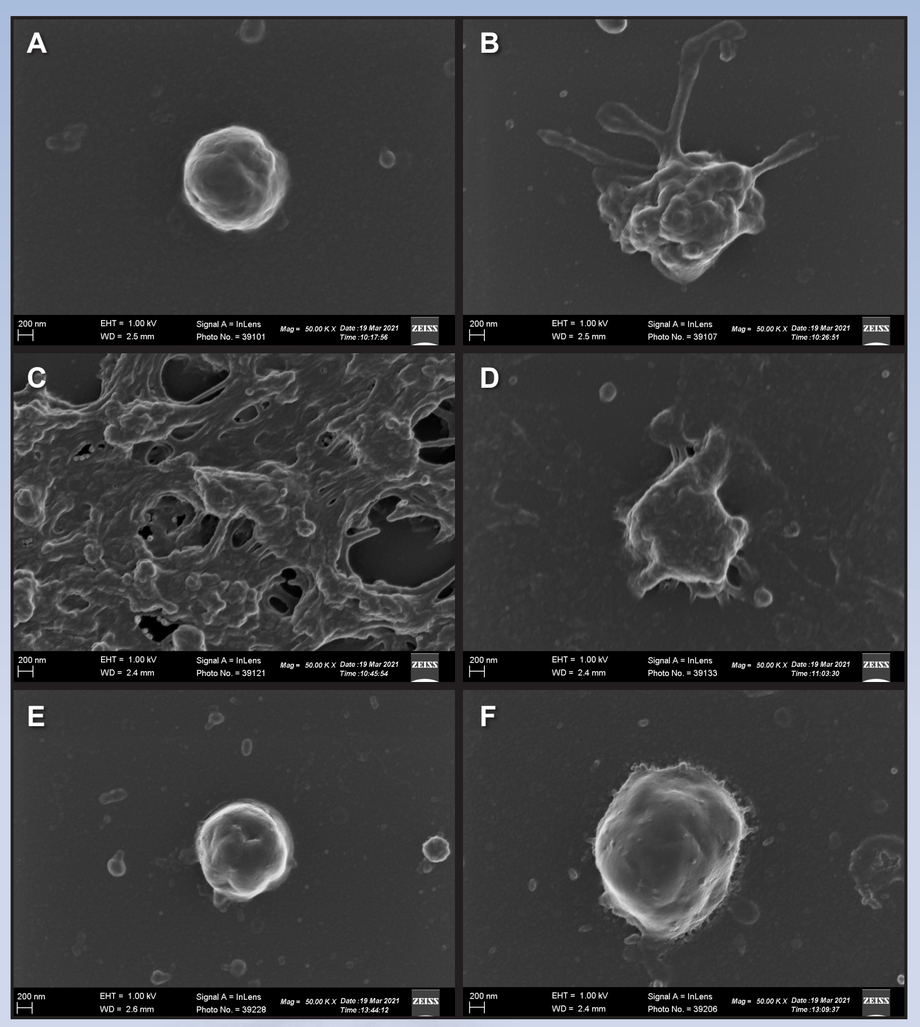

Figure 3: SEM images of platelets of CML patients at time of diagnosis (A and C), after 6 months of treatment with Imatinib (B and D) and control participants (E and F).

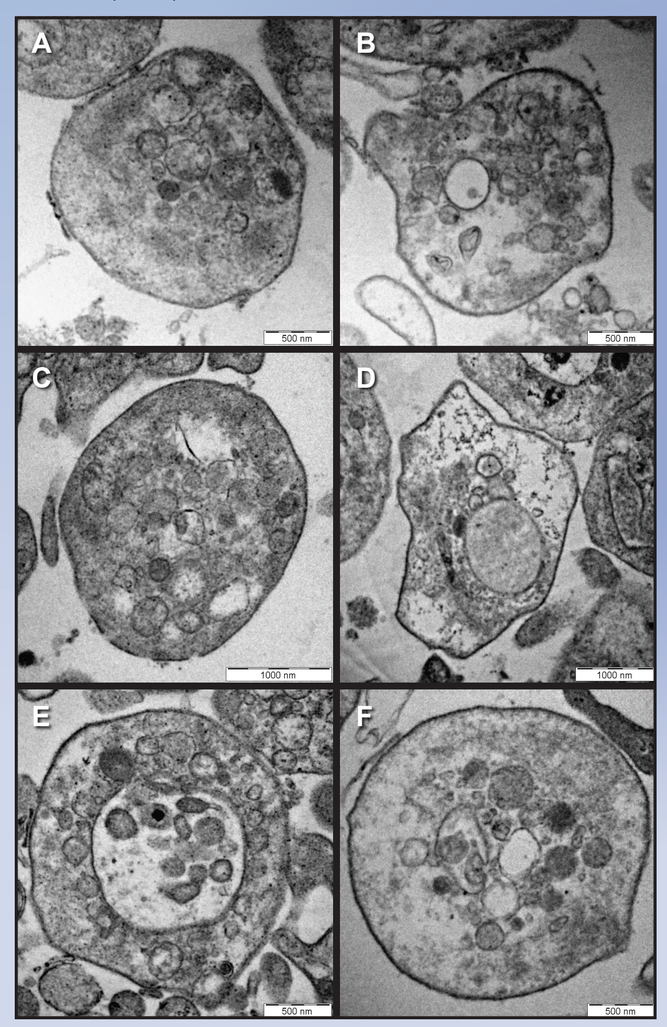

Figure 4: TEM images platelets of CML patients at time of diagnosis (A and C), after 6 months of treatment with Imatinib (B and D) and control participants (E and F).

Selected references
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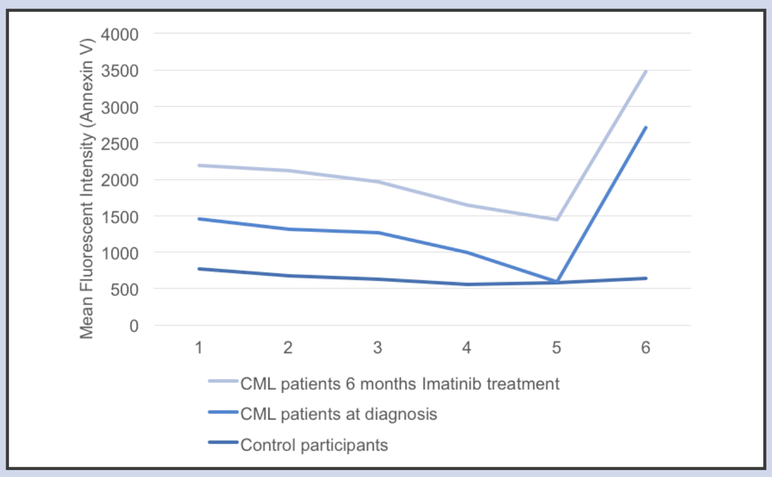

Figure 5: Line graph comparison of mean fluorescent intensity of CML patients at diagnosis, after 6 months of treatment with Imatinib and control participants with the Annexin-V FITC marker measuring apoptosis.

Discussion and Conclusion

Platelet activation in patients with CML is reported to be associated with increased thrombosis. However, the effect of Imatinib on platelets of CML patients is not well understood. Platelets counts and specifically thrombocytosis is widely reported in CML patients; in the current study these reports are corroborated. The current study reports on a statistically significant decrease in platelet counts after treatment with Imatinib for 6 months which can partly be attributed to the increase in platelet activation and apoptosis detected, corroborated by SEM and TEM. This study highlights the fact that platelets are integral to CML pathogenesis and that treatment with Imatinib affects the mechanisms of platelet activation and apoptosis, decreasing platelet counts and increasing the chances for thrombolic events as a result of impaired platelet function.

Abnormalities in platelet functioning observed in this study may partly be due to clonal proliferation of haematopoietic cells in CML patients, specifically of megakaryocyte precursors, as well as the inhibition of platelet tyrosine kinases and the inhibition of platelet-derived growth factor due to Imatinib treatment which disrupts normal homeostasis of platelets. However, the exact mechanism of Imatinib-related platelet dysfunction is not fully understood and warrants further investigation.

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