Ex vivo platelet morphology assessment of chronic myeloid leukaemia patients before and after Imatinib treatment

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Abstract

Chronic myeloid leukaemia (CML) is a myeloproliferative disease and the first line treatment is through the administration of Imatinib, a first generation tyrosine kinase inhibitor. Thrombocytosis and bleeding irregularities are common in CML, however, the morphological variations in CML patients' platelets are not well documented.

In this study, *ex vivo* platelet morphology of control participants, as well as CML patients was assessed before and after Imatinib treatment. The topographical and structural morphology of platelets was determined via scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Qualitative data of SEM and TEM revealed that CML patient's platelets were prone to aggregation and coagulation at time of diagnosis; the samples that were not aggregated at time of diagnosis showed normal discoid shaped platelets which was comparable to control participants' platelets. TEM results of CML patients' platelets at diagnosis showed that internal granular constituents including dense bodies were decreased in comparison to control participants. In all CML patients, platelets appeared activated after 6 months of treatment with Imatinib with membrane structure abnormalities and constituent variations.

Research to date has primarily focused on the effects of CML on leukocyte populations, however, the results of the current study implicate the impact of CML pathogenesis on platelets, seemingly as a result of alterations in normal hematopoiesis. In addition, the impact of Imatinib treatment on platelet morphology was also established, indicating an increase in platelet activation. Recognising and understanding the impact of CML disease progression on platelets is of importance to aid improved patient treatment.

Research Highlights

In the study, results from SEM and TEM indicated that CML patient's platelets were prone to aggregation at time of diagnosis, and activation after Imatnib treatment. Platelet samples that did not aggregate had decreased internal granular constituents.

Keywords: chronic myeloid leukaemia, Imatinib, platelets, morphology, scanning electron microscopy and transmission electron microscopy

1 Introduction

Chronic myeloid leukaemia (CML) is a myeloproliferative disease characterized by the presence of the Philadelphia chromosome (Ph). The Ph chromosome results from the translocation of the *Abelson murine leukaemia viral oncogene homolog 1* (ABL1) gene from chromosome 9 and the *breakpoint cluster region protein* (BCR) gene from chromosome 22 resulting in the formation of the BCR-ABL1 fusion gene (Deininger *et al*., 2021; Hochhaus *et al*., 2020).

The first line treatment of CML, especially in third world countries such as South Africa, is through administration of first generation tyrosine kinase inhibitors (TKIs), such as Imatinib. Imatinib is an inhibitor of the *BCR-ABL1* fusion protein, BCR-ABL tyrosine kinase (Louw *et al.,* 2011). In patients who are unresponsive to Imatinib treatment, second generation TKIs are recommended including Dasatinib, Nilotinib and Bosutinib (Minciacchi, Kumar and Krause, 2021). Second generation TKIs have increased potency compared to Imatinib, improved inhibition against Imatinib-resistant BCR-ABL mutations and quicker molecular responses (Breccia and Alimena, 2014; Oehler, 2020).

The incidence of primary and secondary *BCR-ABL1*-dependent and -independent resistance to Imatinib has been on the increase (Minciacchi, Kumar and Krause, 2021). The mechanisms of *BCR-ABL1*-independent resistance include factors such as bioavailability of the TKI drugs. *BCR-ABL1*-dependent mechanisms of resistance involve increased expressions of the *BCR-ABL1* fusion protein or mutations in the *BCR-ABL1* kinase domain. Primary Imatinib resistance has been reported to occur in 2% to 13% of CML patients and secondary resistance occurring in up to 18% of CML patients as indicated by cytogenetic and hematologic relapse (Mauro, 2006).

CML disease progression is divided into three disease phases including the chronic phase, accelerated phase and blast phase. Symptoms of CML patients range widely between asymptomatic to fever, fatigue, weight loss and splenomegaly (Minciacchi, Kumar and Krause, 2021). These symptoms are usually present in the initial chronic phase of CML and the majority of patients are diagnosed in this phase of CML disease progression (Faderl *et al*., 1999; Goldman and Melo, 2003; Hehlmann, Hochhaus and Baccarani, 2007; Hoffbrand, Higgs, Keeling and Mehta, 2016; Sawyers, 1999). As the disease progresses from the initial chronic phase to the advanced blast phase or crisis,

symptoms in CML patients become severe and include bone pain and severe bleeding (Minciacchi, Kumar and Krause, 2021). The blast crisis of CML is characterised by an increase in immature myeloid or lymphoid blasts thought to be due to the activation of the beta-catenin signalling pathway in granulocyte-macrophage progenitors in CML patients.

In various cancer types, platelet count abnormalities have been frequently noted. Specifically, thrombocytosis in myeloproliferative disorders has often been related to poorer clinical outcomes in several cancer types which has been directly associated with haemorrhagic and thrombotic events. Thrombocytosis and bleeding irregularities are common in CML patients (Lakhotia, Pahadiya, Prajapati, Choudhary, and Gandhi R, 2015). These platelet abnormalities are frequently quantified as part of standard laboratory diagnostic tests (full blood counts) in CML patients, however, the topographical and structural morphological variations in platelets of CML patient's platelets are not well documented.

Platelets are anucleate cells derived from the fragmentation of the cytoplasm of megakaryocytes in the bone marrow (Wojtukiewicz, Sierko, Hempel, Tucker, and Honn, 2017). They are biconvex discoid discs when inactivated and include open canalicular systems, an intricate array of membranes which communicate with the extracellular space, α-granules, dense granules, lysosomes and mitochondria (Buys and Pretorius, 2012; Latger-Cannard, Fenneteau, Salignac, Lecompte, Schlegel, 2013; Pretorius, 2012;). Platelets' main function is to maintain hemostasis, thrombosis and wound healing through platelet activation and the release of granules (Holinstat, 2017; Parise, Smyth and Coller, 2001).

Although the main function of platelets were originally reported to be haemostasis and thrombosis as mentioned; this has evolved over the years to include angiogenesis, metastasis and inflammation (Wojtukiewicz, Sierko, Hempel, Tucker, and Honn, 2017). Various pro-inflammatory factors which are recruiters and activators of leukocytes aiding in platelets immune regulating function are released from platelets upon activation which include CXC chemokine ligand (CXCL)-1, CXCL4, CXCL5, CXCL7, CXCL12, interleukin (IL)-8 and transforming growth factor (TGF)-β. TGFβ is a known immunosuppressive cytokine resulting in tumours and tumour cells from escaping recognition and apoptosis by the immune system (Olsson and Cedervall,

2018). In addition, the release of these factors recruit leukocytes to primary and metastatic tumour sites and promote metastasis through formation of neutrophil extracellular traps (Olsson and Cedervall, 2018).

Platelets' implication in cancer-associated inflammation, thrombosis and metastasis has become a focus for research through targeting of platelets for the possible treatment of cancer (Elaskalani, Berndt, Falasca, and Metharom, 2017; Meikle *et al*., 2017). Anticancer effects of platelet affecting drugs such as heparin and aspirin has been reported. The active ingredient of aspirin, acetylsalicylic acid, is known to inhibit cyclooxygenase (COX)-enzymes, specifically COX-1 from platelets, which are responsible for the formation of thromboxane A2 resulting in the inhibition of platelet activation and aggregation and therefore has been postulated to have an anticancer, protective effect (Olsson and Cedervall, 2018). Platelet-affecting drugs therefore present a significant target as a combined anticancer treatment with conventional cancer treatments such as chemotherapy, radiation therapy, or immunotherapy.

In this validation study the *ex vivo* platelet morphology of control participants, as well as chronic myeloid leukaemia patients was assessed before and after Imatinib treatment by means of scanning electron microscopy and transmission electron microscopy. Research to date has primarily focused on the effects of CML on leukocyte populations, however, the current study implicate the impact of CML pathogenesis and Imatinib treatment on platelets. It is therefore of importance to elucidate the impact of CML disease progression and treatment on platelets to aid improved patient treatment.

2 Materials and Methods

2.1 Collection of blood

Blood samples of 30 healthy volunteers and 6 CML patients at diagnosis and after six months of treatment with Imatinib were collected after an 8-hour period of fasting between 08:00-09:00 AM. Control participants were included in the study if they complied with the following inclusion criteria: aged between 20-60 years, female and male participants, non-smoking, not on any chronic medication. Control participants were excluded according to the following exclusion criteria: chronic or acute illnesses,

autoimmune diseases, hereditary diseases, hypertension, hormonal contraceptive use, smokers and use of platelet affecting drugs i.e. aspirin.

CML patients were included in the study according to the following inclusion criteria: newly diagnosed with CML in the chronic phase of the disease, treated with Imatinib for six months at 400 mg/day (which is the standard dosage for newly diagnosed patients) daily, adult participants aged 18 years and older, female and male participants. Exclusion criteria for CML patients were as follows: human immunodeficiency virus (HIV) positive patients, patients using platelet-affecting drugs (antiplatelet drugs) i.e. cyclooxygenase-1 inhibitors (aspirin), adenosine diphosphate (ADP) receptor antagonists (clopidogrel and prasugrel) and integrin αIIbβ3 (GPIIb-IIIa) receptor blockers (abciximab, eptifibatide, and tirofiban). All CML patients included in the study achieved major molecular response after 6 months of treatment with Imatinib.

Whole blood of control participants was collected in ethylenediamine tetra acetic acid (EDTA) tubes as per standard diagnostic blood drawing procedure by the Department of Haematology, University of Pretoria, Pretoria, South Africa. Blood of CML patients was collected by medical doctors at the Steve Biko Academic Hospital and the Department of Haematology, University of Pretoria, Pretoria, South Africa at diagnosis and after 6 months of treatment with Imatinib.

Platelet-rich plasma (PRP) was obtained from whole blood by centrifuging the blood at 12 300 xg for 2 min and collecting plasma from the separated blood. PRP samples were stored in 1 ml eppendorf tubes within 5 min of separation by freezing at -70°C (Li, Bertino, Coburn and Kuter, 2000).

2.2 Materials

EDTA tubes and needles were acquired from Transpharm (Gauteng, SA). Microplates (96 well) were obtained from Separation Scientific (Randburg, Johannesburg, SA). Phosphate-buffered saline (PBS) was purchased from Gibco-BRL (Invitrogen, Carlsbad, CA, USA) and prepared as a tenfold concentrated stock solution consisting of 80 g/l NaCl, 2 g/l KCl, 2g/l KH_2PO_4 and 11.5 g/l Na_2PO_4. The latter was prepared with double distilled water (ddH_2O) and the pH adjusted to 7.4. A 1×-solution of PBS

was prepared with ddH_2O as a 1:10 dilution of the 10×-stock and subsequently autoclaved (120˚C, 15 psi, 20 min) before use. All blood-contaminated waste materials were collected and discarded into 5 litre biohazardous waste bins.

2.3 Microscopy

2.3.1 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to view the topography of samples with the use of high-energy electrons which bounce off the surface of the solid coated specimen surface to present a high-quality image (Goldstein *et al.,* 2017).

Ex vivo samples were prepared on glass plates with 10 µl platelets per glass plate (Pretorius, 2007). Glass plates with *ex vivo* samples were placed in 6 well plates and left to dry slightly for 1 min or until a film has formed over the sample after which the samples were washed for 20 min in a 50% PBS: 50% distilled H_2O solution (Pretorius, 2007). Samples were fixed with gluteraldehyde and PBS for 30 min and washed 3 times in PBS for 3 min each for subsequent secondary fixation in osmium tetraoxide for 15 min. Samples were washed 3 times each for 3 min and dehydrated for 3 min each in increasing concentrations of ethanol (30%, 50%, 70%, 90%) and three times in 100% ethanol. Samples were critically dried, mounted and carbon coated. High-quality images of morphology of platelet samples were captured with the Zeiss ULTRA plus FEG-SEM (Carl Zeiss (Pty) Ltd, Johannesburg, South Africa) (Pretorius, 2007) at the Microscopy and Microanalysis Unit of the University of Pretoria, Pretoria, South Africa.

2.3.2 Transmission electron microscopy

TEM allows for the visualization of ultracellular structures of platelets. The platelet specimen is illuminated by an electron beam under high-vacuum conditions; the resultant transmitted or diffracted beam magnifies the image up to a million times (Kikuchi and Yasuhara, 2012). The contrasted visualisation of platelets' structure with the use of TEM is as a result of the differences in electron density of the platelet constituents (Kikuchi and Yasuhara, 2012).

Platelets were fixed with a PBS: 2.5% gluteraldehyde (9:1) solution for 45 min at room temperature and then rinsed three times for 5 min each with PBS. Samples were fixed with osmium tetroxide for 15 min and subsequently rinsed three times for 5 min with PBS. Samples were dehydrated for 3 min in increasing concentrations of ethanol (30%, 50%, 70%, 90%) and three times in 100% ethanol. Samples were then infiltrated and embedded with 100% epoxy resin (Hurbain and Sachse, 2011). Ultrathin sections of samples were prepared using a microtome and were contrasted using 4% uranyl acetate for 5 min and Reynolds' lead citrate for 2 min, rinsed with double distilled water and viewed with a Multi-purpose Philips 301 TEM (Electron Microscopy Unit, University of Pretoria, South Africa).

2.4 Statistics

The study was designed to firstly establish reference values for the outcome parameters in healthy volunteers and secondly for chronic myeloid leukaemia patients where changes from baseline with respect to the outcome parameters following 6 months treatment with Imatinib were analysed. The number of subjects included in this study was corroborated by Prof Becker, a statistician at the University of Pretoria, Pretoria, South Africa. Qualitative data capturing included SEM and TEM images and were confirmed by quantitative data (previously published) (Repsold *et al*., 2021).

3. Results

3.1 Scanning Electron Microscopy

With the use of the SEM, the surface topography (structure and morphology) of platelets can be viewed. An electron beam is probed and focused on the sample surface of the prepared platelet sample by a set of lenses in the electron column (Vernon-Parry, 2000). High-quality, high-resolution images of platelets' surface structures were observed. Figure 1 indicates the morphological structure of CML patient's platelets at time of diagnosis and Figure 2 shows CML patients' platelet structure after 6 months of treatment with Imatinib. Control participants' platelets are shown in Figure 3.

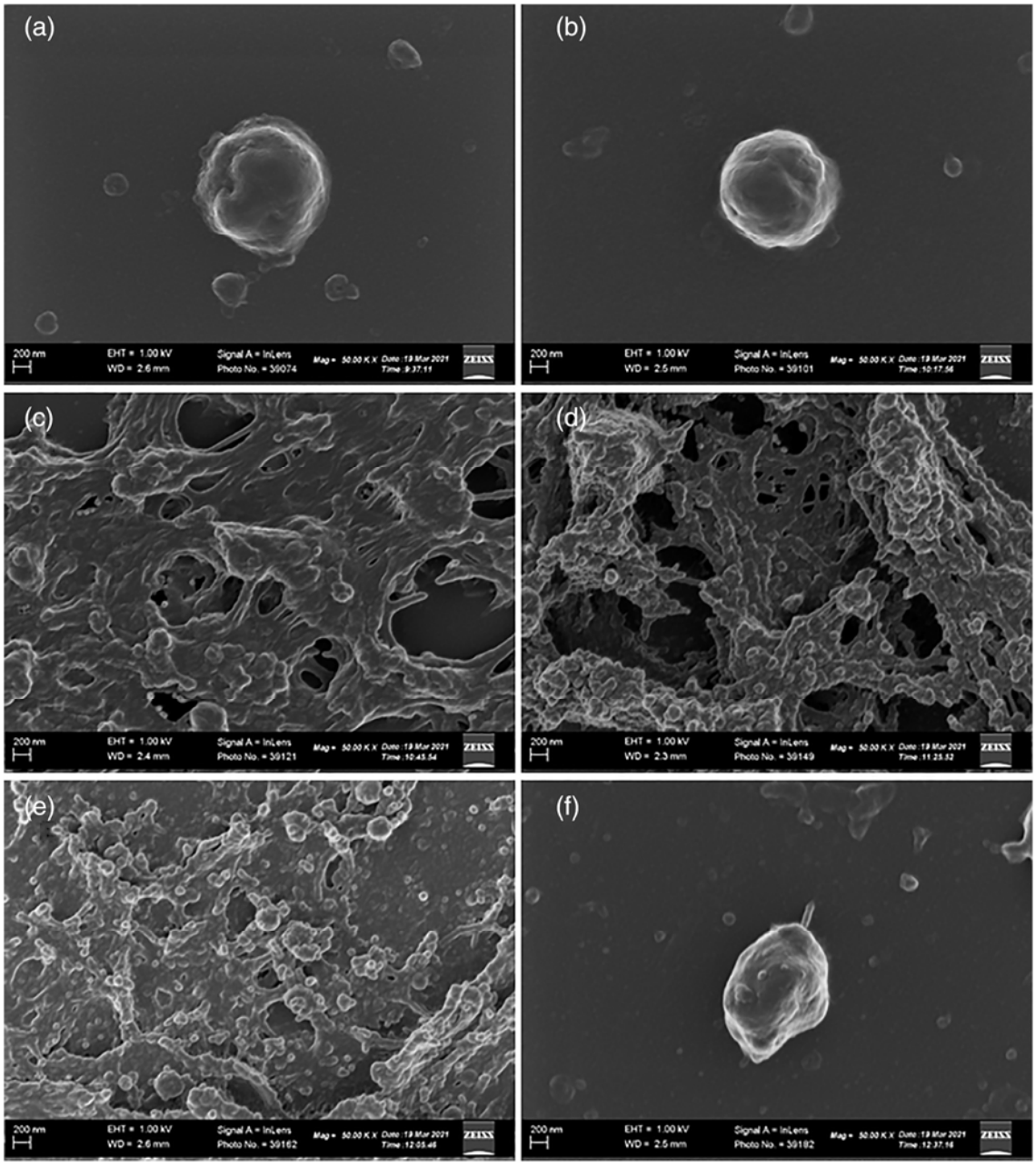

Figure 1: SEM images of platelets of CML patients at time of diagnosis (A – patient 1, B – patient 2, C – patient 3, D – patient 4, E – patient 5 and F – patient 6). Platelet morphology at time of diagnosis indicates normal discoid platelet morphology in A, B and F. Platelet aggregation and coagulation was present in 3 patients' samples at time of diagnosis following sample preparation as shown through spreading and formation of fibrin networks in C, D and E.

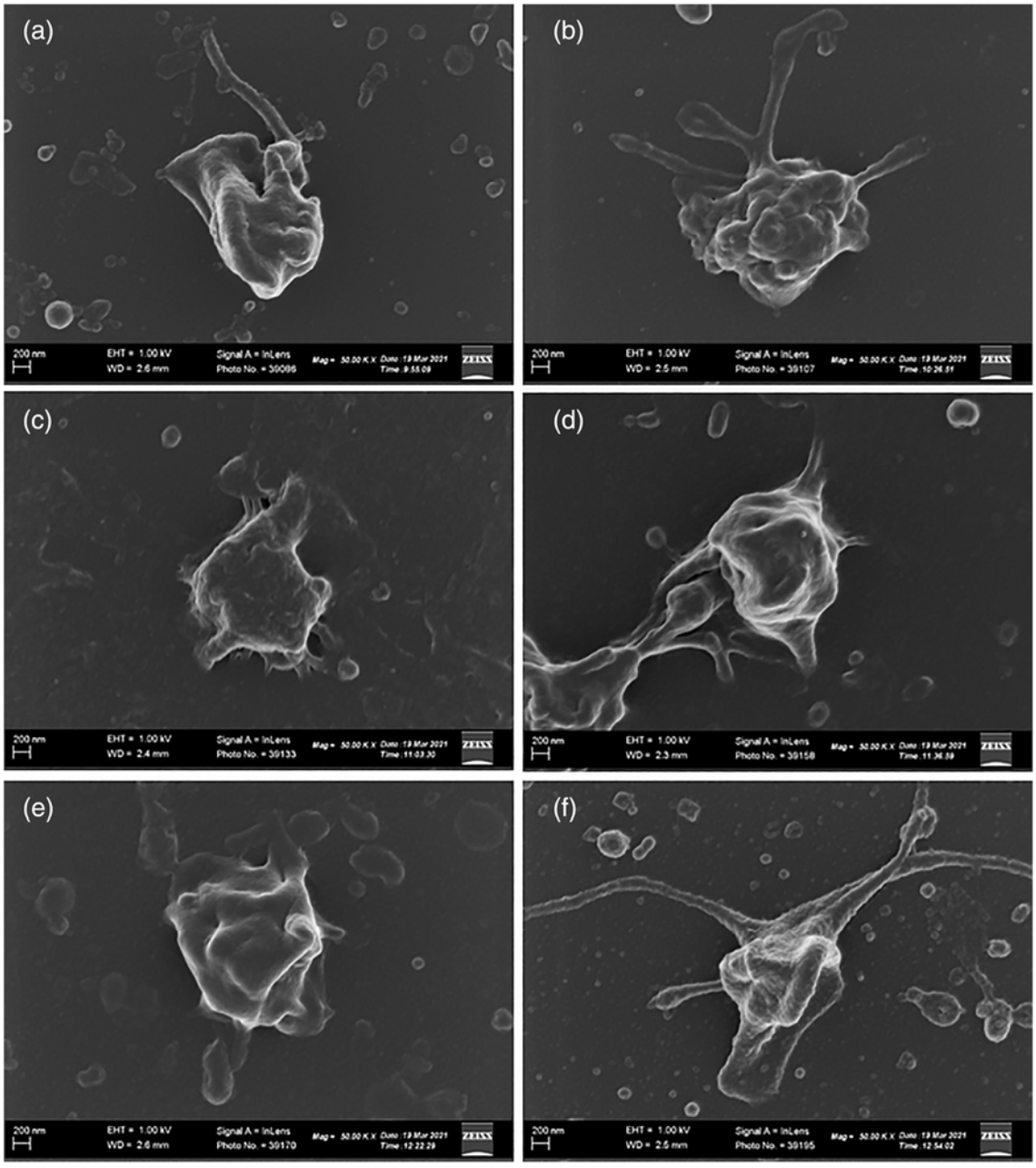

Figure 2: SEM images of platelets of CML patients after 6 months of treatment with Imatinib (A – patient 1, B – patient 2, C – patient 3, D – patient 4, E – patient 5 and F – patient 6). A indicates normal platelet structure lacking signs of activation at time of diagnosis. Platelet morphology in B indicates platelet activation through formation of pseudopodia, spreading of platelets and membrane alterations after 6 months of treatment with Imatinib.

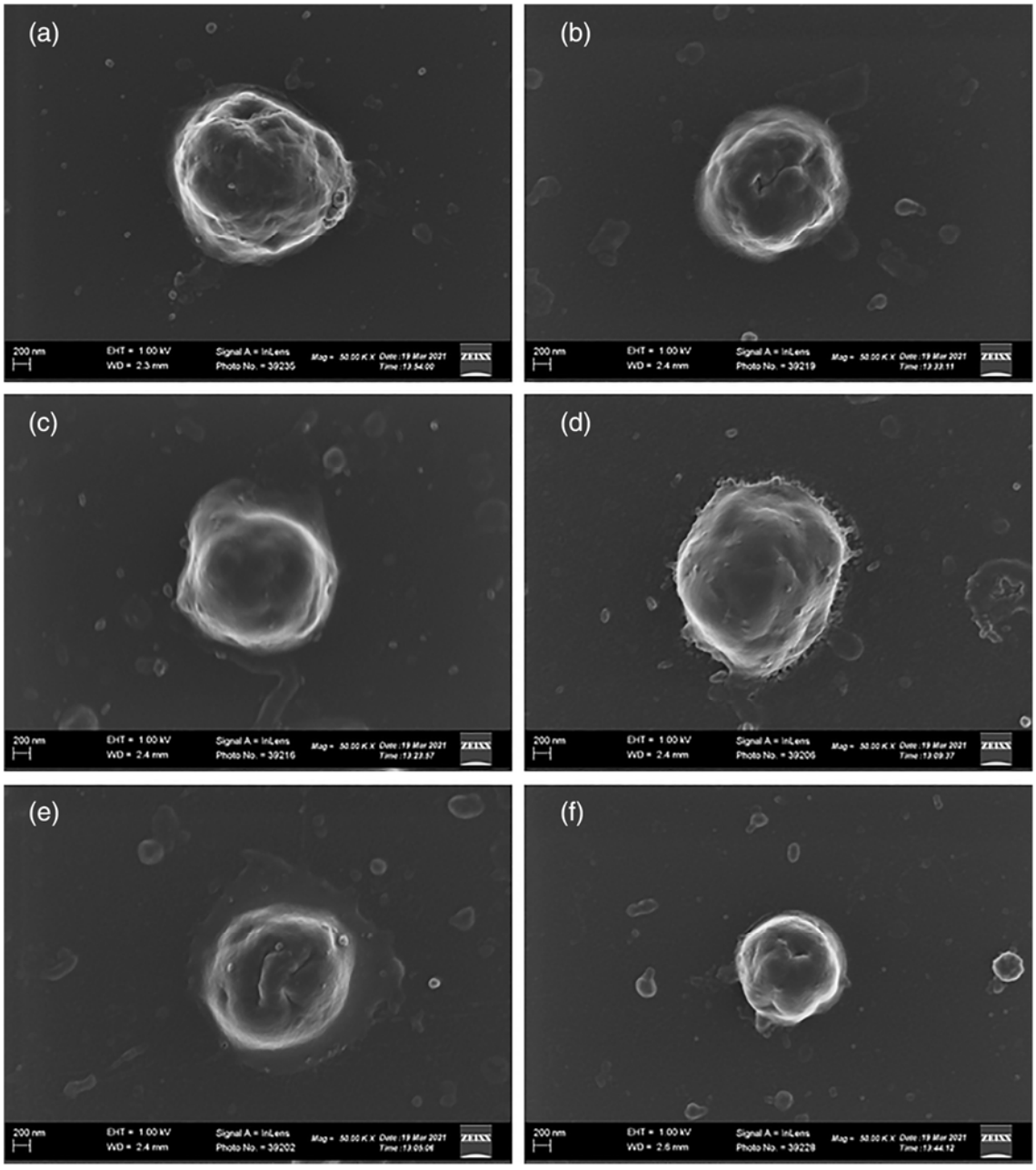

Figure 3: SEM images of platelets of control participants (A-F). Platelet morphology of control participants indicates normal discoid platelet structures lacking signs of activation or aggregation.

CML patients' platelets were prone to aggregation and coagulation at time of diagnosis as seen in Figures 1C to 1E. This is apparent through spreading of platelets and formation of fibrin networks as a result of hyper-activation. Platelet samples of CML patients at diagnosis that did not aggregate (Figures 1A, 1B and 1F), retained their

discoid characteristic shape without activation which was comparable to control participants' platelets (Figure 3). Fifty percent of the patient's platelet samples in this study aggregated during SEM preparation and the other 50% showed normal surface structure of platelets. In all CML patients, platelet morphology appeared activated through formation of pseudopodia, spreading of platelets and membrane surface alterations after 6 months of treatment with Imatinib as shown in Figures 2A to 2F (White and Gerrard, 1978).

3.2 Transmission Electron Microscopy

TEM allowed for the morphological visualization of ultracellular structures of platelets of CML patient's and control participants (Kikuchi and Yasuhara, 2012). Figure 4 indicates the morphological structure of CML patient's platelets at time of diagnosis and Figure 5 shows CML patients' platelet structures after 6 months of treatment with Imatinib. Control participants platelets are shown in Figure 6.

CML patient's platelets were activated and prone to aggregation and coagulation at time of diagnosis (Figure 4D and 4E). This is apparent through formation of giant platelets (Figure 4E) and fibrin networks as a result of platelet aggregation and coagulation of the plasma sample. Furthermore, platelet morphology of CML patients at time of diagnosis (Figure 4A, 4B, 4C, and 4F) revealed characteristic discoid shape of platelets without signs of activation which was comparable to control participant's platelets (Figure 6). The internal granular constituents and dense bodies of CML patients' platelets at time of diagnosis were significantly decreased in comparison to control participants indicating that CML patient's platelets are storage pool deficient at the time of diagnosis. In all CML patients, platelet morphology after 6 months of treatment with Imatinib (Figure 5A to 5F) indicated membrane abnormalities and constituent variations including formation of giant granules, large numbers of lysosomes and increased content of channels of the dense tubular system.

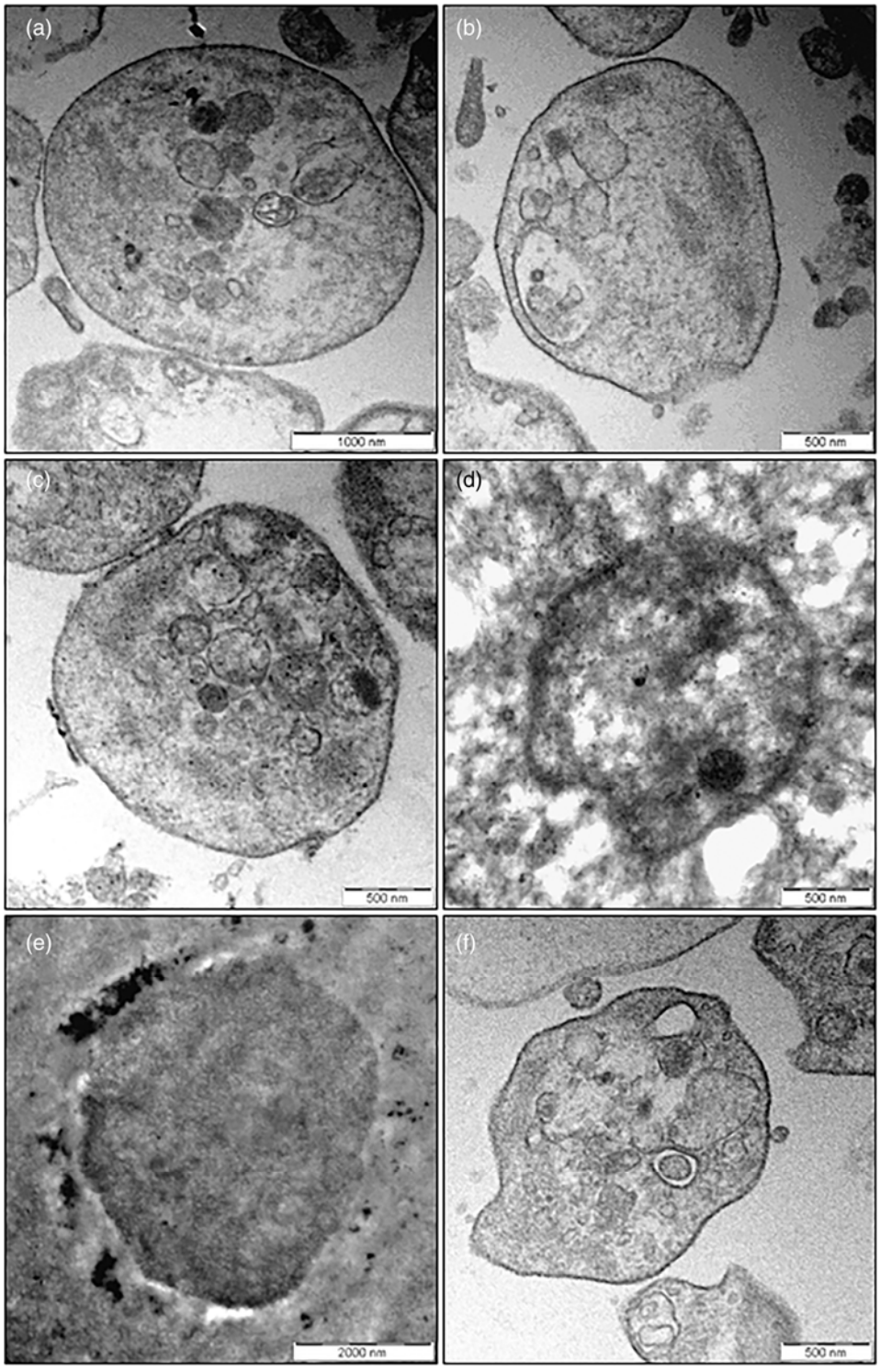

Figure 4: TEM images of platelets of CML patients at time of diagnosis (A – patient 1, B – patient 2, C – patient 3, D – patient 4, E – patient 5 and F – patient 6). A, B, C and F indicates relatively normal discoid platelet structure at time of diagnosis with decreased intracellular granular constituents and dense bodies. Platelet morphology in D and E indicates platelet structures in an aggregated sample at time of diagnosis.

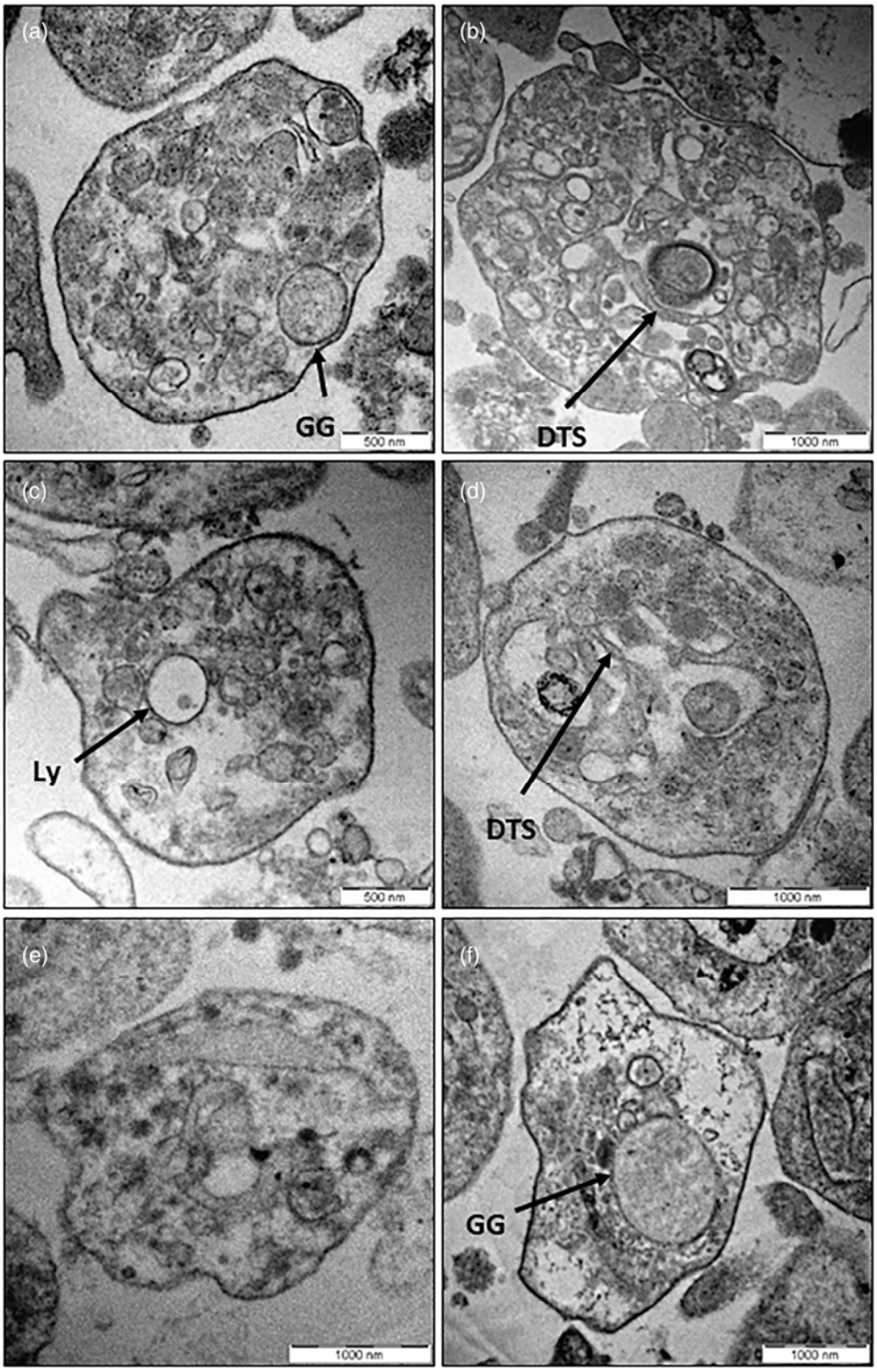

Figure 5: TEM images of platelets of CML patients after 6 months of treatment with Imatinib (A – patient 1, B – patient 2, C – patient 3, D – patient 4, E – patient 5 and F – patient 6). Platelet morphology in B indicates membrane alterations with variations in the normal discoid shape of platelets membranes, increased content of channels of the dense tubular system (DTS), the formation of giant granules (GG) and increased lysosomes (Ly) after 6 months of treatment with Imatinib.

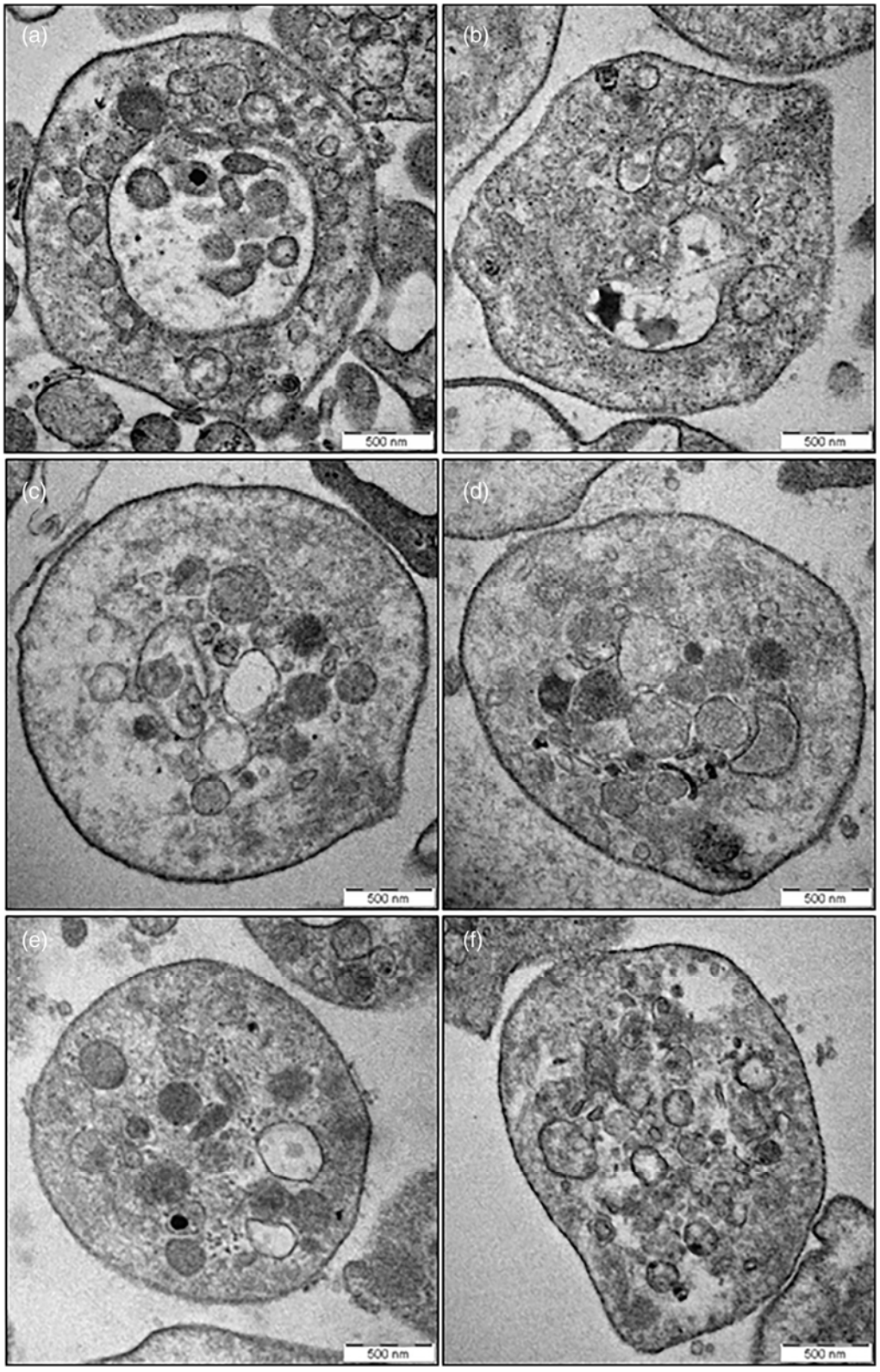

Figure 6: TEM images of platelets of control participants (A-F). Platelet morphology of control participants indicates normal discoid platelet structures with minimal signs of activation and aggregation and normal internal constituents.

4 Discussion

Platelets various functions include hemostasis, thrombosis, wound healing, angiogenesis, metastasis and inflammation which occur through platelet activation and the release of various granules and factors (Holinstat, 2017; Wojtukiewicz, Sierko, Hempel, Tucker, and Honn, 2017). Platelets involvement in tumour progression is as a result of platelet activation and the release of various angiogenic factors including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) from platelet alpha granules stimulating angiogenesis (Olsson and Cedervall, 2018). The various pro-inflammatory factors and cytokines mentioned earlier which are released from platelets recruit leukocytes and further promote angiogenesis through remodelling of the extracellular matrix. Cancer metastasis is directly linked herewith in addition to platelet-linked tumour cell survival in the circulation, extravasation and colonization to a secondary site (Olsson and Cedervall, 2018).

Platelets' are therefore clearly implicated in cancer-associated inflammation, thrombosis and metastasis and their activation is of particular interest as targets for cancer treatment (Elaskalani, Berndt, Falasca, and Metharom, 2017; Meikle *et al*., 2017). The anticancer effects of platelet affecting drugs including heparin and aspirin has been mentioned in literature, in addition to this, various studies in mice have reported that inhibition of platelet activation effectively supresses metastasis. Targeting platelet functioning, including platelet activation and aggregation and subsequent release of platelet granules as an anticancer treatment is therefore of interest in cancer research (Olsson and Cedervall, 2018).

The study of CML pathogenesis has primarily focused on leukocyte populations and limited literature is available of the impact of CML on patients' platelets and further impact as a result of TKI treatment. One study reported on the effects of *in vitro* and *ex vivo* TKI exposure in CML patient's platelet function, it was found that TKI treatment resulted in inhibition of platelet function and stimulated pro-coagulant platelet formation which elucidates the occurrence of bleeding and cardiovascular events due to thrombosis in TKI treated CML patients (Deb *et al*., 2020). It was concluded that there was marked inter-individual differences in response to TKI exposure (Deb *et al*., 2020). The incidence of TKI treatment resulting in impaired platelet function was reported by Ozgur Yurttas and Eskazan to be observed in 29.8% of a population cohort

(Ozgur Yurttas and Eskazan, 2019). In another study by Quintás-Cardama, Han, Kantarjian, and Cortes, it was reported that 66% of the study population presented with Imatinib related platelet dysfunction (Quintás-Cardama, Han, Kantarjian, and Cortes, 2009). Morphological studies on the utilization of SEM and TEM, to our knowledge, has not been extensively reported on in this setting.

In the current study, results from SEM indicated that CML patients' platelets were prone to aggregation and coagulation at time of CML diagnosis of patients in the chronic stage of the disease. This is evident through spreading of platelets and formation of fibrin networks as a result of hyper-activation. Platelet samples of CML patients at diagnosis that did not aggregate, retained their characteristic discoid shape without significant activation which was comparable to control participants' results. This is indicative of inter-individual variations in platelets of CML patient's as 50% of the patient's platelet samples in this study was prone to aggregation and the sample coagulated during SEM preparation and the other 50% showed normal surface structure of platelets. In all CML patients, platelet morphology appeared activated with no aggregation after 6 months of treatment with Imatinib. These results are indicative that treatment with Imatinib in CML patient's increase platelet activation and decrease aggregation. These findings are in agreement with Deb *et al*., whom reported on the inhibition of platelet function following TKI treatment and Sener *et al*., whom reported decreased platelet aggregation in 26% of Imatinib treated CML patient's (Deb *et al*., 2020; Sener *et al*., 2019).

TEM results showed that CML patient's platelets were activated and prone to aggregation and coagulation at the time of diagnosis which was apparent through formation of giant platelets and formation of fibrin networks resulting in aggregation and coagulation of the plasma sample. These results are in accordance with literature which reports that the *BCR-ABL* oncogene and resultant tyrosine kinase proteins produced in CML patients result in platelet aggregation (Deb *et al*., 2020; Oda *et al*., 1996). Furthermore, samples of CML patients at time of diagnosis that did not aggregate showed characteristic discoid shape of platelets without significant activation which was comparable to control participant's platelets as well as SEM results. The internal granular constituents of CML patients' platelets at time of diagnosis were however decreased and showed a significant reduction in the number

of dense bodies in comparison to control participants indicating storage pool deficiency in CML patient's platelets. These results are supported by literature indicating storage pool deficiency in CML patients' platelets through TEM investigation (White and Gerrard, 1978).

In all CML patients, platelet morphology after 6 months of treatment with Imatinib indicated membrane abnormalities with variations in the normal discoid shape of platelets membranes and constituent variations including formation of giant granules, large numbers of lysosomes and increased content of channels of the dense tubular system. These are all indications of platelet activation and inhibition of platelet aggregation due to Imatinib treatment which are in accordance with findings from Sener *et al*. (Sener *et al*., 2019). To our knowledge no literature has reported on the TEM ultrastructure of CML patient's platelets after Imatinib treatment.

The SEM and TEM findings of morphological activation in CML patients after 6 months of treatment with Imatinib in the current study is in agreement with data published by the researchers which indicated that platelet activation in CML patients was significantly increased after 6 months of treatment with Imatinib compared to levels at diagnosis and platelet apoptosis was also found to be increased after 6 months of treatment with Imatinib (Repsold *et al*., 2021). Increased platelet activation and resulting externalisation of phosphatidylserine (PS) is known to be associated with platelet apoptosis and formation of microparticles which were observed in this studies morphological investigations (Repsold *et al*., 2021).

Previous reports on the morphology of platelets in CML patients have indicated that platelets present with storage pool deficiency, reduced granular constituents, especially dense bodies and abnormal aggregation and coagulation responses (White and Gerrard, 1978). These reports were corroborated in the current study with the SEM and TEM results obtained. Storage pool deficiency in CML patients is associated with deficiencies of 5-hydroxytryptamine (5-HT) and ADP which are major consitiuent of platelet dense bodies (White and Gerrard, 1978). These deficiencies result in abnormal platelet aggregation, specifically, a deficiency in ADP results in the inability of platelets to stick together due to failure in transformation of the external platelets surface to a sticky state (White and Gerrard, 1978).

Platelets, and in particular their differentiation from megakaryocytes during hematopoeisis, is impacted by the presence of the Ph chromosome and subsequent formation of the *BCR-ABL1* oncogene due to CML's pathogenesis. This resulted in increased platelet aggregation and decreased activation in CML patients' platelets at time of diagnosis (White and Michelson, 2007). Treatment with Imatinib increase platelet activation which may be attributed to the inhibition of platelets production of tyrosine kinase's and inhibition of platelet-derived growth factor (PDGF) release from platelet α-granules which function to mediate wound healing and repair, affecting normal platelet function and aggregation (Graves, Grotendorst, Antoniades, Schwartz, and Valente, 1989; Pophali and Patnaik, 2016).

5 Conclusion

There has been a marked increase in the incidence of primary and secondary *BCR-ABL1*-dependent and –independent resistance to Imatinib (Minciacchi, Kumar and Krause, 2021; Ning *et al*., 2020). Research to date has primarily focused on the effects of CML on leukocyte populations, however, the results of the current study implicate the impact of CML pathogenesis on platelets, seemingly as a result of alterations in normal hematopoiesis which impact the normal differentiation of megakaryocyte precursors and subsequent differentiation of atypical platelets. In addition, the impact of Imatinib treatment on platelet morphology was also established, indicating an increase in platelet activation due to treatment. The significant abnormalities found after 6 months of treatment with Imatinib may be relatively attributed to the inhibition of platelet tyrosine kinases and disruption of the normal homeostasis of platelets.

Platelet activation is integral to inflammation and thrombosis and mediates various pro-cancerous effects through the release of various soluble mediators from platelet granules. The cross-talk between inflammation, cancer and platelets is therefore an ideal target for research and treatment strategies through antiplatelet therapy. Despite the knowledge implicating platelets in these mentioned processes, there is, nevertheless, limited literature available on the involvement and impact of platelets in many diseases, including CML. This cross-talk or overlap between platelets' functions

in various diseases' pathogenesis stands to be understood to aid clinicians in developing individualized treatment options. Due to the fact that platelets play an important role in inflammation, cancer and tumor development, their role and potential influence in cancer progression is of clinical significance and warrants further investigation into possible novel therapeutic strategies targeting platelet function to improve patient outcomes.

Authorship

Lisa Repsold was responsible for conducting all experiments, analysing data, literature review and the main contributor to drafting of the manuscript. Roger Pool, Mohammed Karodia, Gregory Tintinger and Anna Margaretha Joubert assisted in drafting of the manuscript. All authors read and approved the final manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

Ethical clearance was obtained from The Research Ethics Committee, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa which complies with International Committee on Harmonization of Good Clinical Practice (ICH-GCP) guidelines and Declaration of Helsinki (Ethics clearance number: 284/2015). Control participants and CML patients gave their full informed consent prior to their inclusion in the study and blood collection.

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