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## RETROSPECTIVE DATA ANALYSIS OF ALL REQUESTS FOR FLOW CYTOMETRIC IMMUNOPHENOTYPING IN A TERTIARY HOSPITAL SETTING

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### ABSTRACT

Flow cytometry is a globally accepted diagnostic tool used for the rapid identification of cells based on their surface and intracellular antigens, especially for the diagnosis of haematological malignancies. The aim of this study was to evaluate the requests received for flow cytometric immunophenotyping and to create a profile of diagnoses. In 2014 data regarding indications and diagnoses were captured from request forms received and final diagnosis reports issued by the Tshwane Academic Division (TAD) of the National Health Laboratory Service (NHLS). A total of 1234 requests were received over the one year period, of which 80.4% were performed and 16.8 % were rejected. The most common indications were leukaemia, lymphoma and cytopenia. Nineteen percent of requests received contained no correct indication or clinical history. In total, 103 and 153 diagnoses were established based on peripheral blood and bone marrow aspirate specimens respectively. Samples were mostly rejected due to sample clotting, electronic gate keeping rules and receiving the specimen more than 24 hours after collection.

### KEYWORDS

Flow cytometry, immunophenotyping, diagnostic profiles

### INTRODUCTION

Immunophenotyping is a diagnostic tool used for the identification of cells on the basis of the antigen specific location on the surface of the cell.<sup>[1]</sup> Flow cytometry has been described by Craig and Foon<sup>[2]</sup> as an “indispensable tool”. Advances in computational technology and the availability of fluorochromes in the past fifty years have led to the large scale adoption of this technique in clinical diagnoses.<sup>[2-4]</sup> Flow cytometry is one of the most popular techniques for the diagnosis of haematological malignancies.<sup>[5]</sup> Possessing the ability to analyse 100 000 cells per second, flow cytometry is a high throughput technique from which results can be rapidly obtained.<sup>[6]</sup> In 2008, the World Health Organisation stipulated that flow cytometric results should be used in the classification of acute leukaemia.<sup>[7]</sup> Not only is flow cytometric immunophenotyping vital in the classification of haematologic malignancies, but it is imperative in determining prognosis, monitoring disease progression and therapy evaluation. Due to its high sensitivity, flow cytometry plays an important role in the detection of minimal residual disease (MRD), where residual cells can be identified and monitored to detect relapse of disease.<sup>[5,7]</sup>

Flow cytometric immunophenotyping has had an impact in clinical diagnosis of haematological malignancies and disorders. Examples include foetal blood quantification, HIV patient monitoring, disease staging and patient response to drugs/therapies. It has also had an impact in the immunophenotyping of leukaemia and lymphoma, assessment of platelet function,

structure and defects, as well as assisting in monitoring apoptosis, chemotherapy management and pre-transplant reactions to name a few.<sup>[8,9]</sup>

Annually, the NHLS Department of Immunology at the TAD, Pretoria, receives a large number of requests for immunophenotyping. Although flow cytometry is a popular diagnostic tool in haematology, it remains a costly especially in terms of the high number of requests received at the TAD. Therefore, it is important to establish whether the test is being utilized efficiently at this laboratory. Fluorochrome conjugated antibodies in immunophenotyping accounts for the high cost factor of flow cytometry. It is recommended that multiple antibodies be used in a panel as aberrant markers may occur from time to time. Multiple antibody panels provide more information than a single antibody.<sup>[10]</sup> Each fluorochrome conjugate in a panel could cost approximately R200. Up to twenty conjugates might be required per panel for some flow cytometric tests, depending on the specific examination. Different test panels such as screening panels, lineage specific panels and diagnostic panels are used. The costs of tests could range between R3655.88 and R4974.07 per patient. It is important that flow cytometry is requested for the correct indications to prevent wastage of resources.

The indications for flow cytometric immunophenotyping as recommended by the 2006 Bethesda International Consensus<sup>[11]</sup> included possible signs and symptoms of: cytopenias, elevated leukocyte counts, observation of atypical cells or blasts in bodily fluids, plasmacytosis or monoclonal gammopathy, organomegaly or tissue masses and patient monitoring.

In cases where patients present with non-specific signs and symptoms, causes other than haemato-lymphoid neoplasms must first be excluded. Once these have been ruled out, flow cytometry may then be indicated for the work-up of these patients. Flow cytometric immunophenotyping is indicated in the instances of anaemia, leukopenia or thrombocytopenia. Pancytopenia is regarded to be more likely associated with neoplasia, therefore flow cytometric immunophenotyping is indicated. However, all lineages should be evaluated to determine the origin, as any haematolymphoid neoplasm might cause cytopenia. For example, elevated leukocyte counts may be an indication for flow cytometric immunophenotyping because they are associated with chronic myeloid leukaemia (CML) and chronic lymphoproliferative disorders. Flow cytometric immunophenotyping is unlikely to yield useful information in the cases of neutrophilia with the absence of blasts, isolated polycythaemia, thrombosis and basophilia. The presence of blasts in the peripheral blood or

bone marrow is a definite indication for flow cytometry. Plasmacytosis and monoclonal gammopathy have also been mentioned as indications for flow cytometric immunophenotyping, as it might assist in the detection of abnormal plasma cells and in the classification of multiple myelomas. Organomegaly, lymphadenopathy and tissue infiltrates are considered to be indications for flow cytometric immunophenotyping as it is effective in the diagnosis of tissue-based lymphoid neoplasms. Indications for flow cytometry in patients, who have already been diagnosed with haematological neoplasms include: disease staging, detection of potential therapeutic targets, therapy response assessment, disease acceleration and prognostication.<sup>[11]</sup>

The aim of this study was to describe the number and type of diagnoses made, as well as indications for flow cytometric immunophenotyping, requested at the Tshwane Academic Division (TAD) of the National Health Laboratory Service (NHLS) over a period of one year.

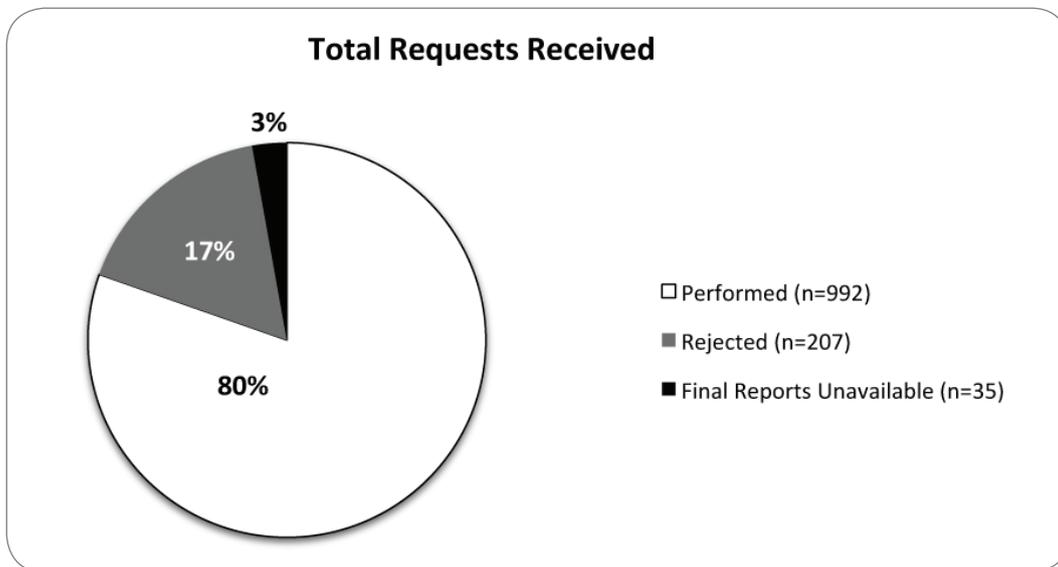


Figure 1. Percentage distribution of the total requests

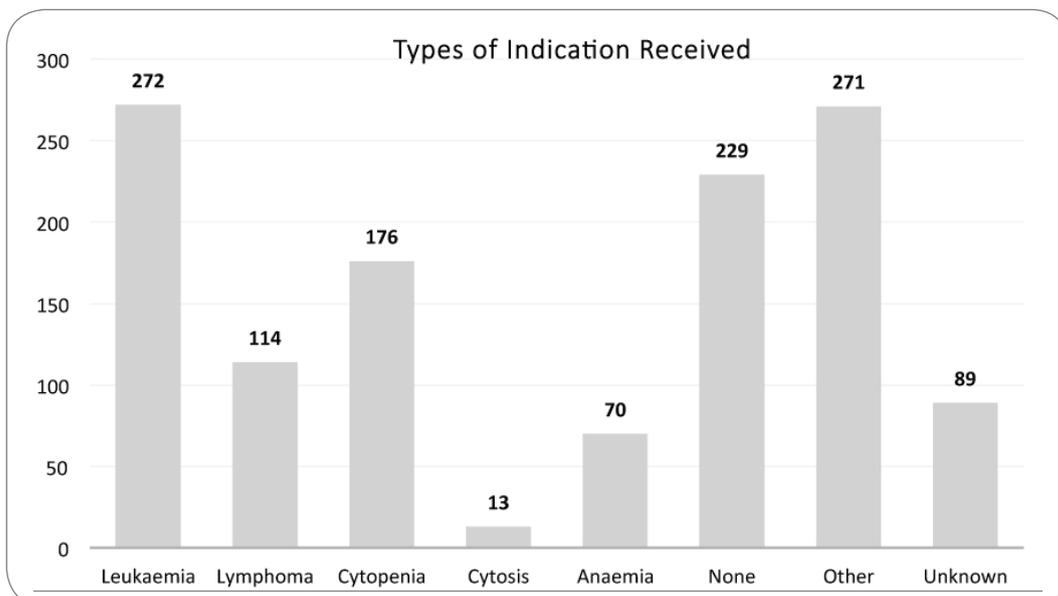


Figure 2. Grouping of requests for flow cytometric immunophenotyping according to indication

**MATERIALS AND METHODS**

Data was gathered from different sources, including all request forms received by the Department of Immunology, TAD of the NHLS, for flow cytometry during the period of 1<sup>st</sup> January 2014 to 31<sup>st</sup> December 2014, a list of rejected requests from the NHLS Laboratory Information System (LIS), as well as a database provided by the Department of Haematology. The final diagnosis of all performed flow cytometric immunophenotyping was obtained from the reports that were available for review. Details about the date of performed flow cytometry, requesting hospital, requesting ward, reason for request, age, specimen type received and diagnosis made, were also collected.

Approval to access the flow cytometry data had been granted by the NHLS. Ethics approval was received from the Research Ethics Committee of the University of Pretoria (142/2015).

**DISCUSSION**

Currently there is no published data on the number and indications for flow cytometry requests received in a tertiary academic setting. This data should be made available to monitor the appropriate cost-effectiveness of this test method in diagnostic laboratories.

Most (80.4%) of the requests received for flow cytometric immunophenotyping were actually performed (Figure 1). This exemplifies the large financial implications the Department of Health faced in 2014. If the lowest priced general panel was used for all tests, the costs would have been a minimum of approximately, R3.6 million. In a country where there is great economic pressure, it is important that the healthcare system aims to maintain a balance between service delivery and cost efficiency.

Indications designated on the request forms were categorized into eight groups namely: leukaemia, lymphoma, cytopenia, cytositis, anaemia, none, other and unknown (Figure 2). All requests which indicated suspected leukaemia i.e., acute myeloid leukaemia (AML), CML, acute lymphoblastic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), Hairy Cell Leukaemia, Mantle Cell Lymphoma, relapse of leukaemia, MRD monitoring,

etc. were included in the Leukaemia category (n=272; 22%). Any indication which included various types of lymphomas were all included in the Lymphoma category (n=114; 9%). The Cytopenia category (n=176; 14%) included bicytopenia, pancytopenia and any single cell line cytopenia, such as thrombocytopenia. The category labelled other (n=271; 22%) consisted of all those indications which did not fall into the seven categories already mentioned, and included organomegaly, fever, bleeding, Paroxysmal nocturnal haemoglobinuria (PNH) and multiple myeloma.

The Unknown group category (n=89; 7%) included those specimens rejected for incorrect collection i.e., tube used, insufficient volume and those that were received after 24 hours of collection.

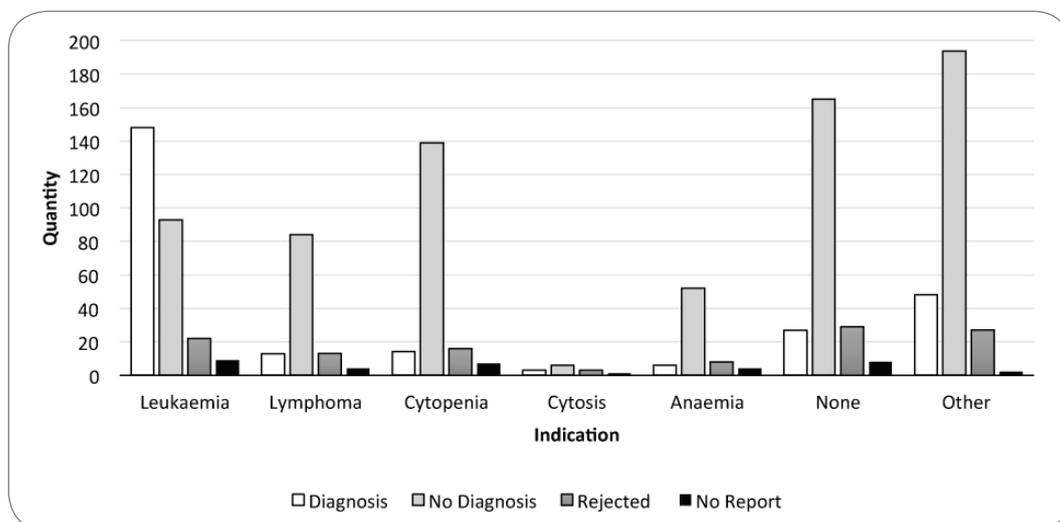
Where a suspected leukaemia or a known leukaemia was indicated on the request form, it assisted in selecting specific panels, thus ensuring the best possible diagnostic results for the patient.

No clinical history or reason for the requests was found in 234 (19%) of the requests received. This could possibly be explained by one of two things: namely the failure to complete the forms with all the necessary information required for performing the laboratory tests or the time constraint during clinical consultations. Additionally, forms lacked clearly designated boxes for clinicians to complete. On 36 forms, the requested tests were written in the space allocated for clinical history. Therefore, no clinical history was provided on these forms.

Focussing on the frequency of diagnosis reported per category, the highest rate was observed in the leukaemia category (n=148; 54%). The lowest rate of diagnosis reported was observed in the category other (n=48; 18%) shown in figure three below.

Surprisingly, in 27 out of the 229 (12%) requests which contained no clinical history or reason for request, a final diagnosis was in fact established. This is important, as even when there was no clinical history, a possible diagnosis could still exist. However, if all requests received are performed, regardless of the indication it may cause a large financial burden.

In total 256 peripheral blood specimens and 730 bone marrow aspirate specimens were received during the year. Diagnosis was established on 103 peripheral blood samples and 153



**Figure 3.** Quantity of diagnoses, rejection and unavailable final report per indication group

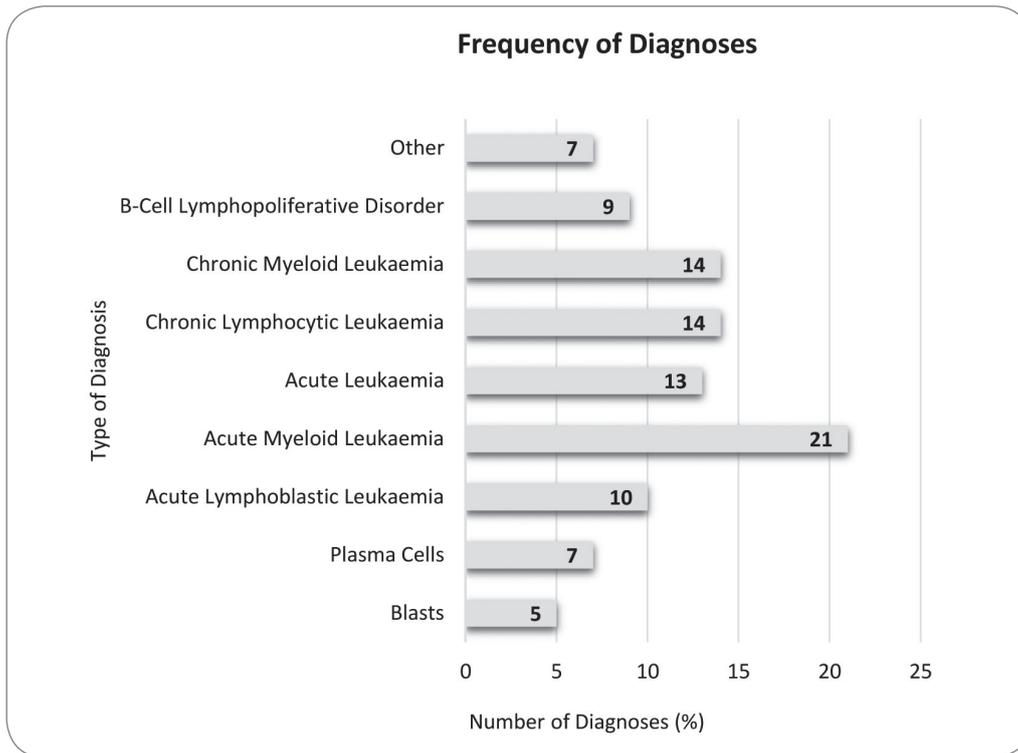


Figure 4. Quantity of diagnoses established per type of diagnosis category

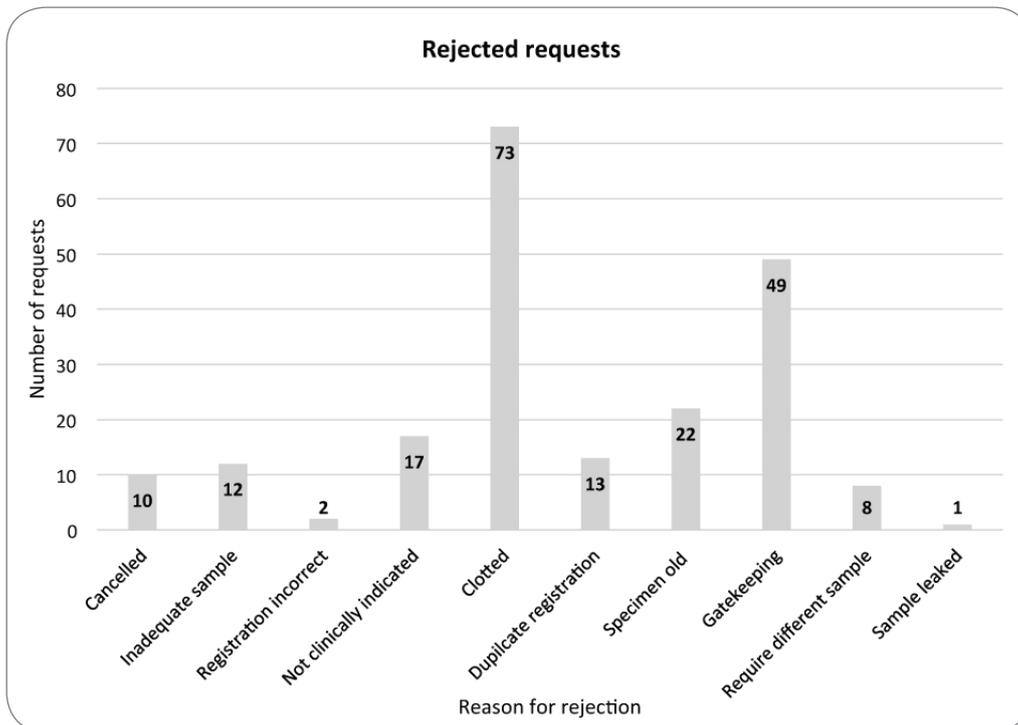


Figure 5. Number of requests rejected and reasons for rejection

bone marrow aspirates. Bone marrow samples are not always necessary in the diagnosis of acute leukaemias and chronic lymphoproliferative disorders, such as CLL.<sup>[12]</sup> Peripheral blood may also be used in the diagnosis of non-Hodgkin's lymphoma (NHL) if there are circulating lymphoma cells.

Regarding final diagnoses established (Figure 4), the greatest number was AML (n=54), followed by CML (n=35), CLL (n=35),

Acute Leukaemia (n=34), ALL (n=26), B-cell lymphoproliferative disorder (n=25), other (n=19), plasma cells (n=17) and blasts (n=12).

The Acute Leukaemia diagnosis category included final reports that indicated a relapse of an acute leukaemia. The B-cell Lymphoproliferative disorder diagnosis category included final reports which established the presence of a B-cell lymphopro-

liferative disorder. However, the particular subtype could not be established with flow cytometry alone. Within the lymphoma category, there were a number of requests that suspected Hodgkin's lymphoma.

Diagnosis of Hodgkin's lymphoma relies on the detection of Reed-Sternberg cells, which are primarily found in the lymph nodes.<sup>[13]</sup> Due to the fact that these cells are rarely located extranodally, flow cytometric immunophenotyping is of limited use.<sup>[14]</sup>

A total of 207 requests received for flow cytometric immunophenotyping were rejected (Figure 5). Most of the rejected requests were observed in the category None (n=29), followed by Other (n=27), Leukaemia (n=22), Cytopenia (n=16) and Lymphoma (n=13) categories (Figure 3). The most common reason for request rejection was due to clotted samples. There were 73 (35%) samples which on arrival were clotted and therefore unsuitable for flow cytometric immunophenotyping.

The adverse clotting of samples during sample collection is mainly attributed to two reasons: i.e., challenging or prolonged venipuncture, or failing to adequately mix the tube after collection ensuring optimal interaction between the blood and anticoagulant.<sup>[15]</sup> The correct ratio of anticoagulant to blood is vital for the integrity of the sample.<sup>[16]</sup>

The gate keeping system of the Steve Biko Academic Hospital rejected 49 (24%) requests. This system has prevented the flow cytometric immunophenotyping from being done, and possibly saved a minimum of R179.138.12 (if only a general screen had been performed).

A cause for concern was the number of requests rejected because they reached the laboratory after the optimal time period of 6-24 hours for processing, had passed. Other reasons for flow cytometry rejection included the following: cancellation by the requesting doctor, inadequate sample (poor cell viability, insufficient sample), incorrect or duplicate registrations of tests and the test was not being indicated clinically, an incorrect sample tube, or that the sample leaked.

## CONCLUSIONS

The results obtained from this study could be used to inform clinicians of the type of requests received for flow cytometric immunophenotyping compared to the proportion which were medically indicated. This would reduce the number of unnecessary requests and preserve funds so that capital may be utilized in a more cost effective manner, thus improving the diagnostic platform. The lack of published flow cytometric data was a limitation to this study. We aim to expand the study to include data from other sites in order to compare our findings. We also hope to establish an overview of flow cytometry statistics in tertiary academic settings throughout South Africa.

## ACKNOWLEDGEMENTS

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