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Recommendations on the use of flow cytometric immunophenotyping for the diagnosis and management of plasma cell dyscrasias.

By the Members of the South African Myeloma Working Group, under the auspices of the South African Haematology Society.

Preamble

Considering the diversity of clinical care options and the frequent restrictions of resources we would like to ensure the accurate diagnosis of malignant plasma cell malignancies. Furthermore we would like to provide information that ensures the optimum management of patients within the region of our healthcare system that they are managed in.

We undertake to do our best to serve all stakeholders in an ethical and professional manner acknowledging our primary responsibility to the wellbeing of the patient.

For clarity we have divided the recommendations into two parts being;

i) Presentation and
ii) Monitoring of response

In each sector we recognize that based on the patients' access to care options, the testing may be restricted by both the availability of multi-parameter flow cytometry and also of different therapeutic options.

Presentation:

In many cases the diagnosis of Myeloma is easily suspected on clinical, biochemical and radiological features and confirmed by proving clonal immunoglobulin and performing a simple bone marrow assessment for plasma cells as below.

International Myeloma Working Group (IMWG) diagnostic criteria are:

	MGUS	**Asymptomatic Myeloma**	**Multiple Myeloma**
Para-protein	<30g/L	>30g/L	>30g/L
BM Plasma cells	<10%	>10%	>10%
End Organ Damage	No	No	Yes

However, specific challenges that the haematopathologists may face are;

1. Initial diagnostic work-up may come from a non clinical haematologist or oncologist
2. The bone marrow aspirate and trephine biopsy (BMAT) is often done before the Serum protein electrophoresis or serum free light chains are performed.

3. The BMAT procedure is invasive, has a threshold cost and the aspirate sample is time sensitive creating a pressure to proceed with the flow cytometry
4. The referring physician doesn't communicate the results of radiological and laboratory (which may have been performed at another service) findings to haematology pathologists.

In counter criticism it is sometimes apparent that;

1. Tests are performed unnecessarily when there is ample evidence of clonality based on the combination of SFLC ratio, high paraprotein level and marked plasmacytosis in the marrow
2. Tests for clonality may be duplicated in different departments in the laboratory (i.e. Immunohistochemistry [R1241], CISH [R4368] on the trephine sent to the histopathologist/haematopathologist whilst flow cytometric immunophenotypic analysis [R3640] is performed by the haematopathologist) .
3. The costs of these tests for private patients often come from the patients "saving component" of their insurance exposing them and their families to additional medical costs, or from their oncology basket affecting the availability of funding for therapy.

Since the pathologists are responsible for both performing the test and requesting the tests, this places them in a position of self-referral. As can be observed, this is not without significant additional cost to the patient/medical insurance fund.

It is our recommendation that the responsibility of compiling the comprehensive clinical, radiological and biochemical features required to make the diagnosis of myeloma, lies with the pathologist. In addition, it is in the best interests of the pathology service to co-ordinate the investigations between departments to prevent the performing of redundant tests.

When a bone marrow biopsy is requested the performance of additional tests [for example immunohistochemistry (IHC), Flow, Flourescent in situ Hybridisation (FISH), Karyotyping) is implicit. However, the consumer protection act requires that the patient has an understanding of the process and the cost consequences. The decision to do further tests should be made taking the above into account and in discussion with the referring physician and patient. The patient needs to be informed of the costs likely to be incurred.

Notwithstanding these requirements, flow cytometry may however have particular value in evaluating malignant plasma cells in specific clinical scenarios, which typically have low tumour burden or poor correlation between clonal protein levels and disease severity.

Examples include:

- MGUS
- Smouldering myeloma
- Non-secretory myeloma

- Solitary Plasmacytoma
- Light Chain Myeloma
- Amyloidosis
- POEMS

In this context (presentation) quantification of plasma cells is based on examination of histological samples. Particularly at presentation, quantification of (high numbers) plasma cells by flow cytometry is considered to be problematic and is not recommended by the International Myeloma Working Group (IMWG) for assessment of disease burden, due to poor sample quality and reduced normal haemopoietic components against which to measure. This can largely be mitigated by sending the "first draw" for flow cytometry and by the use of "whole sample" analysis rather than manipulated or processed samples. Remember that this quality issue affects all quantification tests performed including FISH analysis and karyotyping. Flow cytometry, as in morphology, has the ability to assess normal haemopoiesis and thereby objectively evaluate the quality of the sample and its' representativeness assuming that the sample isn't further manipulated or processed. Furthermore quantification can be aided by examination of the trephine biopsy if it is of reasonable quality.

Regarding the use of flow cytometry, the European Myeloma Network (EMN) makes the following technical recommendations

1) CD38, CD138 and CD45 should all be included in at least one tube for plasma cell identification and enumeration.
2) The primary gate should be based on CD38 vs. CD138 expression;
3) After treatment, clonality assessment is only likely to be informative when combined with immunophenotype to detect abnormal cells.
4) Flow cytometry is suitable for demonstrating a stringent complete remission;
5) For detection of abnormal plasma cells, a minimal panel should include CD19 and CD56. A preferred panel would also include CD20, CD117, CD28 and CD27;
6) Discrepancies between the percentage of plasma cells detected by flow cytometry and morphology are primarily related to sample quality and it is, therefore, important to determine that marrow elements are present in follow-up samples, particularly normal plasma cells in MRD negative cases.

The Euroflow Panels are as follows

Presentation/diagnosis:

Tube 1 CD45 CD138 CD38 CD56 β2 micro CD19 CyIgκ CyIgλ
Tube 2 CD45 CD138 CD38 CD28 CD27 CD19 CD117 CD81

For MRD Euroflow panels are:

Tube 1 CD138 CD27 CD38 CD56 CD45 CD19 CyIgK CyIgλ
Tube 2 CD138 CD27 CD38 CD56 CD45 CD19 CD117 CD81

Backbone markers marked in red are charged only once.

Some centers also use CD 20 and CD200

Monitoring response to therapy:

Once the patient is being managed by a clinical haematologist or oncologist the situation changes regarding the responsibility of the test protocol. The treating physician is in a position to know the patients situation regarding;

1. Additional risk factors (renal dysfunction, cardiac dysfunction, cytogenetic abnormalities, etc.) that may influence therapeutic decisions
2. Previous therapy
3. Serial evaluation of other markers (paraprotein & SFLC)
4. Objective of therapy (degree of response desired)
5. Especially in the context of the next phase of treatment (High dose chemotherapy with autologous stem cell transplant, observation, consolidation, maintenance and even allogeneic transplant decision)
6. What other therapies are available to the patient as determined by their funding situation (State, level of insurance and personal resources)

In this context the treating physician should be responsible to direct the further testing of the patient in communication with the laboratory service. The level of testing should be determined by how the result will influence the next phase of therapy. Obviously, if further therapeutic options are limited then careful consideration should be given to further investigating the patient's disease response.

Where indicated, such as a decision to put a patient on maintenance therapy based on minimal residual disease (MRD) positivity, flow cytometry should be performed and reported as per the EMN recommendations

MRD evaluation requires the use of a 3-laserflow cytometer, which is not available in all sites in SA.

Testing for MRD should fulfill the following criteria for the level of detection and the level of quantification. In addition the report format should reflect these criteria and how the specimen performed.
The MRD level of detection is at least 20 events (malignant plasma cells) in 3 x 10^6 events (bone marrow cells evaluated). This is the limit of detection to reproducibly report **negative** MRD.
The MRD level of quantification is at least 50 events in 5 x 10^6 events. This is the limit of detection to reproducibly report **positive** MRD.

Summary

The objective of this document is to demonstrate an agreement between Clinical Haematologists and Haematopathologists regarding the responsible investigation and reporting of myeloma regarding flow-cytometry on bone marrow biopsies. The technical requirements and reporting format are laid out in the body of the document and the haematopathologists agree to them.

That Haematopathologists will take the responsibility to co-ordinate the initial investigation and to co-ordinate the further testing with the referring physician.

That clinical haematologists and oncologists will take the responsibility to direct the use of flow cytometry to monitor response based on the specific clinical scenario, the treatment plan and therapeutic options available to the patients.