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Critically Appraised Topic

Title: A year with the new WHO 2016 classification: what to expect.

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CLINICAL BOTTOM LINE

Classification: why?

Classification of diseases is part of the language of medicine, categorizing known entities in a way that facilitates understanding between workers in the field and providing a framework for both clinical practice and the generation of new knowledge.

Much of the progress in understanding and management of malignant diseases can be credited to the development and application of classification and staging systems that allow medical investigators to study comparable diseases in comparable patients. When a new classification system (or update such as the new WHO 2016 classification) is presented, it is essential to compare it with the old one in terms of its usability and its usefulness (diagnosis, prognosis). We would address in this work the impact of this new classification for **Myelodysplastic Syndrome (MDS), Myeloproliferative Neoplasms (MPN) and Myeloproliferative Neoplasms/Myelodysplastic Syndrome (MPN/MDS)**.

Available coding systems for the classification of hematological malignancies (interview with Professor Edouard Cornet, Clinical Pathologist in Hematology at CHU Caen, France) and the "MDHW" project in UZ Leuven

Nowadays the available coding systems for hematological pathologies are (i.a.):

- ADICAP
- ICD (ICD-10).

The GFHC (*Groupe Francophone d'Hématologie Cellulaire*) is first hands implied in the elaboration and the update of the ADICAP system (latest update 2013) which is mainly used in French hematological laboratories. In diverse LIS (*Lab Information Systems*) used in France, there is a specific field for that thesaurus. Each hematological disease has a unique code which is commonly used by the different specialists (cytologists, pathologists, …) when they state a diagnosis. Therefore, that makes epidemiologic extraction much easier. One disadvantage of such system is that difficultly includes the newest molecular findings. Also, the latest update of this system was done in 2013 and thus doesn't take into account the new data from the 2016 revision of the WHO classification.

The ICD (*International Classification of Diseases*) classification is maintained by the World Health Organization (WHO) and provides a system of diagnostic codes for classifying diseases, including nuanced classifications. This system is designed to map health conditions to corresponding generic categories together with specific variations, assigning for those a designated code, up to six characters long. The ICD is periodically revised and is currently in its tenth revision (ICD-10: 1994; latest update 2016). The WHO publishes annual minor updates and triennial major updates. ICD-11 was initially planned for 2017 but has been pushed back to 2018.

The on-going project in UZ Leuven MDHW[1] (Multidisciplinary Hematologic Diagnostic Work-up") ["MDHO (MultiDisciplinair Hematologisch Overleg)] was designed with the aim to optimize the hematologic diagnostic process by gathering results from the additional testing (Cytology, Flow Cytometry, Pathology, Cytogenetic & Molecular) in a unique and common report where the final diagnosis is multidisciplinary determined. For its disease's classification, it uses the internationally renowned classification system (WHO classification of tumors of hematopoietic and lymphoid tissues & its ICD10)[2].

CLINICAL/DIAGNOSTIC SCENARIO

The fourth edition of "WHO classification of tumors of the hematopoietic and lymphoid tissues" was issued in 2008. Since then, there have been numerous breakthroughs in the identification of unique biomarkers associated with some myeloid neoplasms and acute leukemia, largely derived from next-generation sequencing (NGS). These advances can significantly improve the diagnostic criteria as well as the prognostic relevance of entities currently included in the WHO 2008 classification and also suggest that new entities should be added. Those changes are at the origin of the new WHO 2016 classification which represents a **revision** of the prior classification rather than an entirely new classification and attempts to incorporate new clinical, prognostic, morphologic, immunophenotypic and genetic data that have emerged since the last edition.

The differences between the WHO 2008 classification and WHO 2016 revision regarding the MDS, MPN & MDS/MPN are highlighted in Appendix 1a.

There are several **nomenclature changes** in this 2016 revision [*4*]:

- Systemic Mastocytosis (SM) is no longer considered a subgroup of the MPN due to its unique clinical and pathologic features (ranging from indolent cutaneous disease to aggressive systemic disease) and now is a separate disease category in the classification.
- The term "Chronic **Myelogenous** Leukemia" was changed into "Chronic **Myeloid** Leukemia".
- In the MDS/MPN category, a provisional entity ("Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T), formerly within the MDS/MPN unclassifiable group, is now accepted as a full entity, now termed **MDS/MPN with ring sideroblasts and thrombocytosis**.
- For the diagnosis and classification of MDS, the revised classification introduces refinements in morphologic interpretation[3] and addresses the influence of rapidly accumulating genetic information. Therefore, the terminology of adult MDS has removed terms such as "refractory anemia" and "refractory cytopenia" and replaced them with "myelodysplastic syndrome (MDS)" followed by the appropriate modifiers: single vs multilineage dysplasia (**SLD / MLD**), ring sideroblasts (**RS**), excess blasts (**EB-1, EB-2**)

[1] *For more information: see also: About the standardization: focus on the MDHW (Multidisciplinary Hematologic Diagnostic Work-up")*

[2] *From discussion with different specialists in Clinical Pathology, no uniform coding system seems to be currently used in Belgium.*

3 (e.g. denominator used for calculating blast percentage is all nucleated BM cells, not just the "nonerythroid cells": most cases previously diagnosed as erythroid/myeloid subtype of acute erythroid leukemia now being classified as MDS with excess blasts)

or the del(5q) cytogenetic abnormality. Interestingly for the childhood MDS, "refractory cytopenia of childhood" remains as a *provisional entity* within this category.

Why an update? Examples of diagnostic criteria changes and their implications

- Myeloproliferative Neoplasms (MPN)

  - Chronic Myeloid Leukemia (CML), BCR-ABL1 +

    In the era of tyrosine-kinase inhibitor (TKI) therapy, newly diagnosed patients for this pathology may have a nearly normal lifespan. But they can also develop a resistance to TKI therapy and therefore regular monitoring for BCR-ABL1 burden and for evidence of genetic evolution is essential to detect disease progression (accelerated phase: AP). The criteria for AP were revised in this WHO 2016 classification and include hematologic, morphologic and cytogenetic parameters which are supplemented by additional parameters usually attributed to genetic evolution (see Appendix 1b). Those "response to TKI therapy" criteria for AP are still now considered as "*Provisional*".

  - BCR-ABL1- MPN (Essential Thrombocythemia (ET), Polycythemia Vera (PV), Primary Myelofibrosis (PMF)

    In recent years, molecular data with demonstrated diagnostic/prognostic importance have emerged, suggesting the need for revision to the previous diagnostic criteria for this sub-group.
    - The discovery of novel molecular findings in addition to *JAK2* and *MPL* mutations, in particular the *CALR* mutation.
    - *CSF3R* mutation: strongly associated with chronic neutrophilic leukemia (CNL).
    - Polycythemia vera (PV): while using the hemoglobin levels (Hb > 18,5 g/dl (♂), > 16.5 g/dl (♀)) published in the fourth edition, was possibly underdiagnosed. Tefferi and Barbui have described patients (*Masked polycythemia vera*) with lower hemoglobin levels (16-18.5 g/dl (♂), 15-16.5 g/dl (♀)) but with mutation in *JAK2* gene and with a bone marrow morphology pathognomonic for a PV [*21*]. Those observations are at the origin of the revised criteria for this pathology (see Appendix 1c).

    Also the persistent controversy regarding the role and inclusion of histopathology for the differentiation between "true" essential thrombocythemia (ET) from prefibrotic/early primary myelofibrosis (prePMF) had been thoroughly described in literature [*37*]. This situation has prognostic implications and is achieved by identifying (among others features) the morphologic findings in the BM biopsy (including the lack of reticulin fibrosis at onset). It has been argued that these criteria showed poor interobserver reliability and are not sufficiently robust enough to allow a clear-cut identification of MPN subgroups. Thiele and al have however achieved a multicenter study to validate the WHO classification for this situation [*37*]. It is also important to always keep in mind that the WHO classification does not claim that a single histologic parameter characterizes a subgroup but that morphologic patterns are very important, only in context with clinical and laboratory findings though [*8*].

- Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)

    - Chronic myelomonocytic leukemia (CMML)

      Recent evidence has shown that a more precise prognostication can be obtained with a blast-based sub-classification: CMML-0: < 2% blasts in PB and < 5% in BM; CMML-1: 2-4% in PB and/or 5% to 9% blasts in BM and CMML-2: 5-19% blasts in PB 10 tot 19% in BM and/or when any Auer rods are present. The revision incorporates the CMML-0 category (absent in WHO 2008 classification) into the classification scheme. Precise morphologic evaluation is essential in view of the importance of separating promonocytes (considered as blasts equivalent cells) from monocytes. The most commonly mutated genes in CMML are *SRSF2, TET2* and/or *ASXL1* (> 80% of cases). Other mutations occur at lower frequency (*SETBP1, NRAS/KRAS, RUNX1, CBL and EZH2). ASLXL1* is a predictor of aggressive disease behavior and has been incorporated into a prognostic scoring system for CMML. *NMP1* mutation is seen in a rare subset of CMML (3%-5%) and has a more aggressive effect on clinical course.

    - Atypical CML, BCR-ABL1 negative (aCML)

      This rare MDS/MPN subtype is now better molecularly characterized and can be more easily separated from CNL (also a rare subtype of MPN) similarly characterized by neutrophilia. As aforementioned, CNL is strongly associated with the presence of *CSF3R* mutation which is very rare in aCML (< 10%). aCML is also associated with *SETBP1* and/or *ETNK1* mutations in up to a third of cases [*4*]). Finally, the driver mutation (*JAK2, CALR, MPL*) associated with the others BCR-ABL1- MPN are typically absent in aCML.

- Myelodysplastic syndromes (MDS)

The same cytogenetic abnormalities listed in the 2008 WHO classification remain MDS-defining in a cytopenic patient, even in the absence of diagnostic morphologic dysplasia [*8*]. Although, for a specific MDS subtype ("MDS with isolated del(5q)), del(5q) remains as the only cytogenetic or molecular genetic defining abnormality but based on recent data showing no adverse effect of 1 chromosomal abnormality in addition to the del(5q) it may also be diagnosed if there is 1 additional cytogenetic abnormality besides the del(5q), unless that abnormality is monosomy 7 or del(7q) [*4*].

Moreover, a large amount of data has recently become available on recurring mutations in MDS. Targeted sequencing of a limited number of genes can detect mutations in 80-90% of MDS patients. The most commonly genes in MDS are *SF3B1, TET2, SRSF2, ASXL1, DNMT3A, RUNX1, U2AF1, TP53* and *EZH2* [*30*]. Though, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in a patient with unexplained cytopenia because acquired clonal mutations identical to those seen in MDS can also occur in the hematopoietic cells of apparently healthy older individuals without MDS ('*clonal hematopoiesis of indeterminate potential*' *CHIP*). Further study is required to investigate possible links between specific mutations and subsequent development of *bona fide* MDS. Further, the number and types of mutations are strongly associated with disease outcome in MDS and the addition of mutation data improves the prognostic value of the existing risk-stratification scores in MDS. *TP53* is associated with aggressive disease in MDS in general.

Concerning MDS with ring sideroblasts (MDS-RS), recurrent mutations in the spliceosome gene *SF3B1* are frequent and associated with the presence of ring sideroblasts. MDS-RS is now included in this 2016 revision and it is largely based on the correlation between ring

sideroblasts and an *SF3B1* mutation which appears to be an early event in MDS pathogenesis and also correlates with a favorable prognosis. Recent studies showed that in case of MDS with RS, the actual percentage of ring sideroblasts is not prognostically relevant [*23*]. Thus, in the revised classification, a diagnosis of MDS-RS may be made if ring sideroblasts are ≥ 5% in the presence of a *SF3B1* mutation (in opposition to ≥ 15% by absence of mutated *SF3B1* gene). The MDS-RS cases lacking *SF3B1* mutation appear to have an adverse prognosis. Although the role of multilineage dysplasia versus the *SF3B1* mutation in influencing outcome in MDS-RS remains controversial.

QUESTION(S)

1) *How could we practically measure the changes and implications regarding the diagnosis (cytology, pathology, flow cytometry, cytogenetic and molecular) that this new WHO 2016 classification involves?*

2) *From an epidemiologic perspective:*
    - *Is the patient's distribution while applying stricto sensu the diagnostic criteria of the WHO-2008 classification the same as while applying those of the 2016 revision?*
3) *Evaluation of cytology and other additional testing (flow cytometry, pathology, cytogenetic & molecular):*
    - *With those new diagnostic criteria and changes within the WHO 2016 classification, what is the decision-making value of the cytology and the other additional testing? Are the molecular and cytogenetic findings brought to light for our patients in accord with the literature? Which prognostic/diagnostic information can we deduce from them?*
    - *How could we evaluate the impact of the quality of the samples on the final diagnostic?*

SEARCH TERMS

1) MeSH Database (PubMed): MeSH term: "Myelodysplastic syndromes"; "Myeloproliferative neoplasms"; "Myelodysplastic syndromes + diagnosis", "Myelodysplastic syndromes + guidelines", "Myeloproliferative neoplasms + diagnosis"," Myeloproliferative neoplasms + guidelines", "WHO-2008 classification myeloid neoplasms", "WHO 2016 classification myeloid neoplasms".
2) PubMed Clinical Queries: MDS rationale changes 2016 WHO classification, MPN rationale changes 2016 WHO classification.
3) PubMed: "MDS + Molecular findings", "MPN + Molecular findings".
4) National Comprehensive Cancer Network, https://www.nccn.org/.
5) UptoDate: http://www.uptodate.com/home

RELEVANT EVIDENCE/REFERENCES

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APPRAISAL

Practical impact of the new WHO 2016 classification

To assess the practical impact of this new WHO 2016 classification, we have met the different interveners of the hematologic diagnostic. Those are Dr. C. Brusselmans (Clinical Pathologist: for Cytology), Pr. Dr. G. Verhoef (Clinician hematologist), Pr. Dr. T. Tousseyn (Pathologist) and Pr. Dr. L. Michaux (Molecular Biologist & Cytogeneticist). The subjects discussed includes the following questions:

- *In general, does this new WHO 2016 classification add/modify something for your daily work?*
  - *Focus on respectively MDS, MPN/MDS, MPN and AL.*
- *Which are, according to you, the most useful information in a cytology rapport?*
- *How is your work influenced by the quality of the sampling?*
- *Standardization of the hematologic diagnostic process.*

The complete transcript of those interviews can be founded in Appendix 2.

The different specialists shared the opinion that practically this new WHO classification have not changed a lot for their daily work. They highlighted the greater importance accorded to the data from cytogenetics and molecular.

Professor L. Michaux insisted on the fact that although this new classification implies more and more the molecular markers, it has also a side-effect of over-consumption of Next-generation sequencing (NGS) (mostly true for extern centers, for which some requests are insufficiently clinically justified). Nowadays, we have to keep in mind that NGS is not yet reimbursed in Belgium. In the present days of harsh socioeconomic conditions, a more clinically-driven prescription for NGS should ideally be applied. Finally, there is still a need of multi-center studies to assess the prognostic/diagnostic signification of the newest described molecular findings.

Moreover, even with regards of the greater importance of molecular findings in this 2016 WHO revision, cytology is still the first step for a hematological diagnostic that can be confirmed by the

genetic data and plays the role of dispatcher for the additional testing. Indeed, a throughout cytological examination of the bone marrow (e.g. assessing dysplastic features for a myelodysplastic syndrome) allows the set-up for additional testing or panels of tests in the other hematological diagnostic sub-disciplines (mainly for flow cytometry, cytogenetic & molecular).

Also, about the impact of the quality of samples:

- The quality of bone marrow samples mostly depends on the technical skills of the doctor performing the bone marrow aspiration/biopsy. That implies an optimal formation for the clinician hematologists interns but also for the interns in laboratory medicine. That's the reason why the bone marrow punctures are now exclusively taught to those two kinds of specialists in our institution. Formerly, any interns in Internal Medicine learned to carry out the aspiration/biopsy and that needed more time and supervision. Also, the nurses who are present in the puncture room have to be optimally trained (e.g. they have to learn to warn the clinicians if there are no bone marrow grains while he can still perform an additional aspiration). Finally, the clinician asserts that it's always useful for an intern to see the results of his aspiration (by examining himself the bone marrow smears) to be aware of the consequences of a non-optimal bone marrow puncture.
- For the pathologist, too often the bone marrow biopsies do not meet the length-criteria mentioned in the guidelines (~ 2.0 - 2.5 cm) [*10*]. That has a great impact as the pathologist has to assess the architecture of the bone marrow. As the biopsy is too limited, it shall mainly reflect the first inter-trabecular spaces (in the sub-cortical layer). Those are physiologically empty and then a too limited biopsy could give the picture of an aplastic bone marrow (*sampling artefact*).

More specifically, about the standardization:

- The cytologists in our hospitals already use the same referential ("Lag Hemato Atlas: https://w1.uzleuven.be//labo/Leermodule/HEMATO_ATLAS/) which allows better standardization for the cytology reports. Especially for the peripheral blood, bone marrow aspiration and body fluids, it specifies the criteria to assess i.a. the dysplastic features and the cellularity for the several cell-lineages.
- The reports for molecular and cytogenetics are also standardized.
- The clinician has proposed that we should implement an informatics tool which obliges the prescriber of a bone marrow aspiration to also prescribe a peripheral blood examination. As we retrospectively evaluated the situation for our cohort of new diagnosed MDS, MPN & MDS/MPN patients (without SM), a peripheral blood examination was missing for approximately 30% (19/64) of bone marrow prescriptions (41.0% (16/39) into the sub-group MPN, MDS/MPN; 11.54% (3/25) into the sub-group MDS).

About the standardization: focus on the MDHW (Multidisciplinary Hematologic Diagnostic Work-up")

MDHW ("MDHO") was implemented in May 2011 in LWS (LIS of UZ Leuven). It regroups into a unique and common report ("MDHO verslag") the following information:

- relevant clinical information
- reports of cytology-pathology-flow cytometry-cytogenetic-molecular
- conclusion of the multidisciplinary hematological staffs ("MOC")
- clinical indication of bone marrow aspiration
- hematological classification of the disease (sub-classification into subgroups of: MDS, MPN/MDS, MDS, Acute Leukemia, Lymphoma).
- ICD-10 code & classification
- diagnostic probability index: scale from 1 to 3: 1 (poor), 2 (moderate), 3 (strong diagnostic evidence).

- differential diagnose (if it is raised following the multidisciplinary hematological process).

It integrates also the possibility to integrate pictures in the LIS. This function can have a lot of applications i.a.:

- illustration and comparison of the pathological and morphological pattern and confrontation with international guidelines and with literature.
- monitoring of the report of morphological abnormalities both on individual and population levels.

The fill-in method is mainly based on "Copy/Paste" of the several reports into specific fields available in the LIS (under the MDHW work-list). This current manual method causes a work-overload: a lot of time and energy were/are put in for the manual introduction of the relevant clinical and diagnostic elements. The data introduction has to be a continuous process to create a reliable database. Consequently, the hematological diagnose work-up requests should be multidisciplinary implemented in the LIS to allow the introduction of standardized conclusions of each testing and clinical information by the different specialists in this common MDHW report. This is primordial to make the global hematologic process more efficient.

Since May 2011, more than 3000 patient's files for bone marrow aspiration were integrated in our coding system. The analyze of this MDHW database provides a large diversity of data's i.a.:

- distribution of diseases by age-group, unit, ...
- diagnostic probability index by pathology
- correlation and discrepancies between the clinical and diagnostic reports.

This broad and detailed database requests from us and from all the implicated hospital units to further investigate the potential bottlenecks that can occur by hematological diagnosis. By doing so, we intend to optimize the diagnose in the daily clinical/diagnostic practice and to come to a better multidisciplinary integration.

Epidemiological distribution of the pathologies according the different classification systems (WHO 2008 classification -WHO 2016 revision)

Materials & methods

Patients:

We performed a retrospective study of the 1658 patients (diagnosis + follow-up) who underwent a bone marrow aspiration during the period between March 2016 and March 2017. We used our homemade MDHW coding system ("MDHO werklijst") and focused on <u>new diagnosis for MDS, MPN, MDS/MPN & SM</u> (and also Acute Leukemia although not discussed in this work). The accuracy of a good disease classification depended on a correlation between two independent observers.

<u>Remarks:</u>

The final diagnosis was sometimes subject to changes during the period of our study (cf. bone marrow of late march 2017 & molecular/cytogenetic TAT (*Turn Around Time*)). The certitude of a 100% accurate classification can thus not be guaranteed. This study allows us to express trends of disease's distribution but does not pretend to be a published material.

Results

The incidence of the different groups of hematologic disorders (MDS, MDS/MPN, MPN & Acute Leukemia) is (relatively) similar year to year (Appendix 3).

MDS (Appendix 4)

As we compare our cohort of patients with a new diagnosis of MDS in 2016 while applying respectively the 2008-WHO and 2016-WHO criteria, we notice the following changes:

- 1 patient classified as "Refractory anemia with ring sideroblasts" moves into the "MDS-RS-MLD" category according to the 2016 criteria.
- For 8 patients with the diagnosis "Refractory anemia with excess blasts", 2 move into the "MDS-EB-2" and the 6 others to "MDS-EB-1".
- 1 patient classified as "Refractory thrombocytopenia" according to the WHO-2016 criteria falls into the denomination "MDS-SLD".
- Our 2 patients with the diagnosis "Refractory anemia" moves into "MDS-SLD".
- For 12 patients with the diagnosis "Refractory cytopenia of childhood or with multilineage dysplasia", 6 are now classified as "MDS-MLD" and the 6 others as "MDS-RS-MLD".

MPN, MDS/MPN (Appendix 5)

For MPN, MDS/MPN and SM, as we compared the patients from our 2014 cohort with those of 2016, the distribution is stable for ET (11 in 2014 and 2016), CML (9 in 2014 versus 8 in 2016) and SM (11 versus 10) and slightly differs for CMML (6 versus 9). Interestingly, in our 2016 cohort, we do not have any new diagnosis of PV which has required a bone marrow aspiration's examination. That can be explained by the fact that a bone marrow aspiration is no more required according the new diagnostic criteria of the WHO 2016 revision as the patient is already known with (all 3): a sustained absolute erytrocytosis (*major criterion 1*), a mutation of *JAK2V617F* or *JAK2* exon 12 (*major criterion 3*) and with a subnormal serum erythropoietin level (*minor criterion*) (see also Appendix 1c).

The distribution into our group of MPN, MDS/MPN & SM (49 patients) is as followed: ET (11), SM (10), CML (9), CMML (9), PMF (Primary Myelofibrosis) (5), aCML (2), MDS/MPN unclassifiable (2), MPN unclassifiable (1) and JMML (1). Even with the reserve imposed by our limited cohort and also that this cohort represents only the patients who had a bone marrow aspiration for their diagnostic work-up, we observe that this distribution is in agreement with the data from the literature (cf. Incidence, survival and prevalence of myeloid malignancies in Europe, O. Visser, European Journal of Cancer (2012) [*40*].

The sub-classification for CMML patients is as followed: 7 with CMML-0 & 2 with CMML-2.

Evaluation of the cytology and the other additional testing (flow cytometry, pathology, cytogenetic & molecular)

Our first aim was to assess this contribution of the additional testing (cytology, cytometry, pathology, cytogenetic and molecular) to the decision-making process regarding the final hematologic diagnosis. For cytology, we wondered if the evoked diagnosis by this initial step of the diagnosis process was changed or not by the data from the other additional testing. For pathology, we wanted to assess the proportion of cases where the bone marrow biopsy is in concordance with the bone marrow aspiration (cytology) but essentially with the final diagnosis.

Then, when a new classification system (in this case the WHO 2016 classification) is presented, it is essential to compare it with the old classification in terms of its usability and its usefulness (diagnosis & prognosis). Therefore, we have chosen to confront the findings in molecular and cytogenetics to what is already known and described in the literature.

MPN, MDS/MPN & SM (n= 49 patients)

- Cytology (Appendix 7)

  The raised diagnosis by this initial step is mainly unchanged for the following entities: CML BCR-ABL1+ and CMML. For both of them (each time for 8/9 patients), the diagnosis was proposed by the cytologists and not changed afterwards. For the other patients (1/9), the diagnosis of a CML or a CMML was in each cases suspected. On the other hand, for ET, PMF and SM, the diagnosis was evoked with certitude by the cytology for respectively 6/11, 1/5 and 2/10 patients and was suspected for 4/10 patients with SM and 1/5 with PMF. Finally, for 3/5 PMF, 5/11 ET and 4/10 patients with SM, the bone marrow aspiration showed too limited determinative features. Further investigations showed that amongst them, the bone marrow aspiration was of poor quality for 2 cases of ET and 2 patients with PMF. For those pathologies, cytology does not seem to be determinative.

- Flow cytometry (Appendix 8)

  We have noticed a large amount requests for flow cytometry in this cohort with exclusion for the patients with SM (36/37 patients). For the great majority of them (29/37; 78%), those requests were canceled following the bone marrow smears examination (insufficient morphological arguments to perform immunophenotyping). The NCNN Guidelines states that flow cytometry is not needed for the diagnosis work-up of patients with suspicion of MPN but only advised when meeting arguments for disease progression to advanced phase (CMML) or AML (PMF) [*3*]. The reason why there are still an important amount of flow cytometry requests in this sub-group in our institution can be explained by the fact that UZ Leuven is an academic hospital and then the place for formation of interns.

  For 3 cases of CMML, flow cytometry was performed and revealed for 1/3 patient (CMML-2) the presence of ~ 20% cells with a monocytoid differentiation. It was inconclusive for the 2 other patients with a CMML diagnosis and also for the unique cases of JMML and MDS/MPN unclassifiable in our cohort. For 1 patient with PMF, flow cytometry was also performed and revealed nothing.

  For the 10 patients with SM, flow cytometry was always performed and confirm the presence of mast cells with expression of CD25 (*minor criterion*).

- Pathology (Appendix 9)

  For CML, BCR-ABL1+, ET, PMF and SM, the pathologist observations are mainly in concordance with the final diagnosis (respectively for 8/9, 9/11, 3/4, 7/10 patients). Pathology, thanks to the bone marrow biopsy, can thus more easily assess the morphologic dysplastic features of ET, PMF and SM. The CMML diagnosis is a little bit more challenging (2 non concordances, 2 differential diagnoses and 5 concordances). The overall concordance percentage for Pathology for the sub-groups MPN and MDS/MPN is 76,74% (33/43 biopsies).

- Cytogenetics (Appendix 10)

  To perform a karyotype in the context of MPN, MDS/MPN, the predilection tissue is bone marrow except for the PMF for which blood is preferred. Interestingly, in our cohort, karyotypes (when requested) were only performed on bone marrow (42 patients). That also means that for the PMF karyotype was never performed on blood as recommended. The proportion of abnormal/normal karyotype is 0.42/0.58. All of our 9 patients with a diagnosis of CML, BCR-ABL1+ had logically the Phi-Chromosome (t(9;22) (q34;q11)); one had

simultaneously a loss of Y. One of our patient with CMML had a 7-/7q- (del(7)(q21q36)) which is described in literature for CMML, two others presented a loss of Y [*15*]. Our only patient with a diagnosis of JMML had a monosomy 7 which is included in the WHO diagnostic criteria for this entity. Our patients with PMF harbored a pseudodiploid complex karyotype (46, sl, t(3;7)(p12;q35)) and the others a pseudodiploid complex karyotype with a deletion of 20q.

- Molecular (Appendix 11)

  The predilection tissue to perform NGS for the MPN, MDS/MPN is also the bone marrow. Practically though there are almost as more requests from peripheral blood (16) than from bone marrow (21). For 10 patients of our cohort, the NGS was simultaneously carried out on bone marrow and blood.

  For our 11 patients with a diagnosis of ET, our molecular findings are in adequacy with what it is described in the literature. Indeed, 90% of them are mutated for one of the 3 driver gene (*JAK2* (6 by NGS and 1 by specific PCR primer for *JAK2 V617F*), *MPL* (1) and *CALR* (2)). One ET patient is finally triple negative. For aCML, our patients harbored no mutations for *JAK2, CALR, MPL* and *CSF3R* but were positive for *SETBP1*, *ASLX1*, *TET2* and *SRSF2* which is also in accord with the literature. For our patients with PMF, only one gene was mutated at time of diagnosis: *JAK2* (which is mainly mutated (2 patients)), then *CALR* (1 patient) and finally *U2AF1* (1 patient). This distribution is also in accordance with the literature. For CMML a diverse variety of mutated gene was found. Those respected also the data from literature as the most mutated genes are *SRSF2* (4 patients (~ 50% of patients with CMML in literature)), *TET2* (4 patients, (~ 60% in literature)), followed by *ASLX1* (3 patients (40% in literature)) and *RUNX1* (2 patients, (15% in literature)) [*15*]. Finally, also 2 patients had mutations into *NRAS/KRAS* which are also described in literature [*15*].

MDS (n= 25 patients)

- Cytology (n = 25 patients) (Appendix 12)

  In general, for our cohort of patients with a positive diagnosis of MDS, the correct diagnosis (even with the accurate sub-classification) was achieved for 60% of cases (15/25). Although a MDS was risen into a differential diagnosis for 8/25 of our patients (30%). This score can be explained by the fact that diagnosis of MDS is essentially based on a morphological assessment and that there are few pathognomonic dysplastic features for this diagnosis. Eventually, often a MDS is a diagnosis *ter exclusionem.* Despite the minimal diagnostic criteria established in 2007 by the "International Consensus Working Group", the diagnosis of a MDS can still be subjective in particularly for the patients with early low risk disease [*44*]. Some authors evaluated that diagnostic discrepancy at the time of initial presentation up to 20% of patients [*43*]. The problem mainly occurs with patients without excess blasts where diagnosis is based solely on dysplasia.
  On the other hand, for the MDS-RS-MLD, the cytology gave the diagnosis for 5 out of the 7 patients.
  For the last 3 patients of our cohort for which the diagnosis of MDS could not be achieved by the cytological step, 2 of them presented a bone marrow aspiration with poor quality.

- Flow cytometry

  Flow cytometry was performed in our cohort of patients with MDS for 5/25 patients and confirmed a population of myeloblasts (3.0% for 1 patient with MDS-EB1; 6.0 and 7.0% for 2 patients with MDS-EB-2) and showed an abnormal B-lymphocyte population for 1 patient with MDS-SLD and 1 with MDS-MLD.

- Pathology (n= 22 patients) (Appendix 13)

  The overall concordance percentage for Pathology for MDS was ~90% (~ 68% full concordance (13 patients) = MDS and subtype (e.g. MDS-EB-1)) **+** ~ 32% partial concordance (6 patients): MDS without accurate subtype). The diagnosis was not concordant for ~10% of our patients. The evaluation of the dysplasia (except for the megakaryocyte's lineage) remains a challenge for the pathologists. Therefore, in situations of MLD, the pathologist observations were discordant for a patient simultaneously for MDS-MLD and for MDS-RS-MLD and the sub-classification was also not possible for 2 patients with MDS-MLD, 1 with MDS-RS-MLD and 2 with MDS-SLD.

- Cytogenetics (n = 25 patients) (Appendix 14)

  The karyotype was abnormal for 9 patients of our cohort (36%). This is a little bit below what the literature has described (cohort of 2124 patients (*Haase&al.*) but our cohort of patients with new diagnosis of MDS was very limited [*41*]. A "complex karyotype" was found for 2 patients with MDS-EB-1, 1 with MDS-EB-2 and 1 with MDS-MLD. 1 patient with MDS-RS-MLD had for his part a complex monosomal karyotype. It is well-established that complex karyotypes are associated with a poor prognosis [*6*]. The 2 others patients of our MDS-MLD sub-group have a del(20q) and a loss of Y which are abnormalities associated with a good prognostic [*6*].

- Molecular (Appendix 15)

  In concordance with what is described in the literature, 95% of our patients (18/19 tested) with a diagnosis of MDS have a mutated gene targeted by NGS [*4*]. The frequency of the mutated gene respected what is stated in the literature with the exception of *SF3B1* which is less frequently mutated (7.69%) in our cohort [*2*], [*29*], [*4*]. That could be explained on one hand by the limited number of tested patients (19), on the other hand by the fact that one of our patient with a diagnose of MDS-RS-MLD died before that NGS could be initiated. The found mutations play also a prognostic role: most of them cause a poor prognostic (*BCOR, U2AF1, TP53, SRSF2, DNMT3A, ZRSR2, RUNX1, ETV6, STAG2, EZH2* and *IDH2*) compared to the only know favorable mutation in our MDS cohort: *SF3B1*.

Impact of the quality of the bone marrow aspiration/biopsy on the final diagnostic

In the last part of our evaluation, we evaluate the impact of bone marrow aspiration/biopsy with a defective quality to the final diagnosis.

- Transversal glance

Primary myelofibrosis is naturally the entity causing most of difficulties to the cytologists mainly because of a poor quality of the aspiration (*dry-tap*) which is common for this disease. For each of our patients with a diagnosis of PMF (5), the bone marrow aspiration was of poor quality. Fortunately, the pathologists at the same time received a biopsy of good quality (4/4 biopsy for this indication). For cytogenetic, karyotype was also with poor quality for 3/5 patients. A transversal glance at the influence of poor quality (for cytology and cytogenetics; the biopsy was not performed) could explain a lower diagnostic probability index in the MDHW for a patient with PMF.

- Specific examples

We already discussed the impact of a sub-optimal bone marrow aspiration on the cytological diagnosis of MDS (2/3 patients with a final diagnose of MDS had a suboptimal aspiration and therefore

no cytological determinant diagnostic was achieved) and of ET (2 patients with a final diagnose of ET had a poor quality aspiration and therefore no pathognomonic features were present on bone marrow smears). Also for SM, the quality of the biopsies was non-optimal for 3/10 patients and for two of them, the diagnosis raised by the pathologist was in discordance with the final hematological diagnosis.

CONCLUSION/COMMENTS

Standardization of the hematological diagnosis remains an arduous process. For example, the diagnosis of MDS is essentially based on an attentive examination of the dysplastic features on bone marrow smears which can ultimately give arguments to perform next-generation sequencing. On the other hand, the sole presence of a mutation in a MDS-associated gene (without dysplastic features) cannot definitively confirm this diagnosis. This mutation has to be interpreted in a multidisciplinary spectrum; in correlation with the data from clinic (cytopenia), cytogenetic and also dysplastic cytological features. Similarly, for MPN & MPN/MDS, some mutations can be found in the different MPN/MDS without specificity and cytological, pathological and clinical findings can help for sub-categorization into MPN or MPN/MDS. Moreover, WHO criteria are guidelines and early-stage diseases can present themselves with an incomplete part of the WHO criteria (e.g. morphological pathognomonic findings of CMML with an absolute monocytosis below the WHO diagnostic criterion ($< 1.10^9$/L). Besides there are different means implemented in our institution ("UZ Leuven Hemato Atlas", "MDHW report") to achieve a more standardized elaboration of additional testing reports. Moreover, the quality of the bone marrow aspiration remains primordial as poor-quality samples directly influences the final diagnosis.

The new criteria introduced by the 2016 WHO revision may at first sight not literally change the routine work for the hematological laboratories (cytologists, pathologists, cytogeneticists and specialists in flow cytometry) but the newly discovered mutated genes and the new sub-classification of MDS allows a better risk stratification of the patients and higher likelihood of the final diagnose. However, multi-center studies evaluating the prognostic/diagnostic impact of those new molecular findings remain essential and a more clinically and multidisciplinary justified request for next-generation sequencing should take place.

The multidisciplinary oriented approach used in the WHO 2016 revision to classify the MDS, MPN/MDS and MPN is one of the arguments proving that the on-going project MDHW in UZ Leuven has all its meaning in times when the clinic data as well as the findings from additional testing have to gather to establish a definitive hematologic diagnostic.

TO DO/ACTIONS

- Further development of LIS software: standardized tools available for the clinicians allowing multidisciplinary additional testing requests.
- Giving the opportunity to the clinicians to prescribe additional testing in accordance with the pathology suspicion. Moreover, integrate a informatics tool to ensure that each time a bone marrow is prescribed, also a peripheral blood examination would be carried out.
- Consequently, computationally automatize the MDHW work-list ("MDHO werklijst"). The different conclusions of the additional testing should be automatically filled in.
- Use more a step-wise/cascade process for the hematological diagnostic: each specialist (cytologists, pathologists, ...) should themselves introduce their diagnostic probability index and the relevant elements in the MDHW ("MDHO werklijst") to potentially suggest which additional testing should be required and finally to multidisciplinary establish the final diagnostic.
- Integrate an informatics tool (like "MDHO") more globally (nationally) to facilitate the generation of epidemiologic data and to allow more easily multi-center studies.

APPENDIX

Appendix 1: a) WHO 2016 revision for classification of Myeloproliferative neoplasms (MPN), Myelodysplastic/Myeloproliferative neoplasms (MDS/MPN) and Myelodysplastic syndromes (MDS) (differences with the fourth edition (WHO 2008) are highlighted in yellow).

Table 1. WHO classification of myeloid neoplasms and acute leukemia

WHO myeloid neoplasm and acute leukemia classification
Myeloproliferative neoplasms (MPN)
Chronic myeloid leukemia (CML), *BCR-ABL1*$^{+}$
Chronic neutrophilic leukemia (CNL)
Polycythemia vera (PV)
Primary myelofibrosis (PMF)
PMF, prefibrotic/early stage
PMF, overt fibrotic stage
Essential thrombocythemia (ET)
Chronic eosinophilic leukemia, not otherwise specified (NOS)
MPN, unclassifiable
Mastocytosis
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia (aCML), *BCR-ABL1*$^{-}$
Juvenile myelomonocytic leukemia (JMML)
MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
MDS/MPN, unclassifiable
Myelodysplastic syndromes (MDS)
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood

b) Criteria for the accelerated phase (AP) of CML

Table 2. Criteria for CML, accelerated phase

CML, accelerated phase criteria	
Any 1 or more of the following hematologic/cytogenetic criteria or response-to-TKI criteria:	
• Persistent or increasing WBC (>10 × 10^9/L), unresponsive to therapy	**"Provisional" response-to-TKI criteria**
• Persistent or increasing splenomegaly, unresponsive to therapy	• Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response* to the first TKI) or
• Persistent thrombocytosis (>1000 × 10^9/L), unresponsive to therapy	• Any hematological, cytogenetic, or molecular indications of resistance to 2 sequential TKIs or
• Persistent thrombocytopenia (<100 × 10^9/L) unrelated to therapy	• Occurrence of 2 or more mutations in *BCR-ABL1* during TKI therapy
• 20% or more basophils in the PB	
• 10%-19% blasts† in the PB and/or BM	
• Additional clonal chromosomal abnormalities in Ph^{+} cells at diagnosis that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2	
• Any new clonal chromosomal abnormality in Ph^{+} cells that occurs during therapy	

Large clusters or sheets of small, abnormal megakaryocytes, associated with marked reticulin or collagen fibrosis in biopsy specimens may be considered as presumptive evidence of AP, although these findings are usually associated with 1 or more of the criteria listed above.

*Complete hematologic response: WBC, <10 × 10^9/L; platelet count, <450 × 10^9/L, no immature granulocytes in the differential, and spleen nonpalpable.

†The finding of bona fide lymphoblasts in the blood or marrow, even if <10%, should prompt concern that lymphoblastic transformation may be imminent and warrants further clinical and genetic investigation; 20% or more blasts in blood or BM, or an infiltrative proliferation of blasts in an extramedullary site is CML, blast phase.

c) Comparison between diagnostic criteria for PV in the 2008 WHO classification (left) and in the 2016 revision (right) (differences are highlighted in yellow).

Table 3. Criteria for polycythemia vera (PV)

Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria:

Major criteria

1. Hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume*
2. Presence of *JAK2* V617F or other functionally similar mutation such as *JAK2* exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro

*Hemoglobin or hematocrit > 99th percentile of method-specific reference range for age, sex, altitude of residence

or hemoglobin > 17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from a person's baseline value that cannot be attributed to correction of iron deficiency

or elevated red cell mass > 25% above mean normal predicted value.

Table 4. WHO criteria for PV

WHO PV criteria
Major criteria
1. Hemoglobin >16.5 g/dL in men
Hemoglobin >16.0 g/dL in women
or,
Hematocrit >49% in men
Hematocrit >48% in women
or,
increased red cell mass (RCM)*
2. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion†

*More than 25% above mean normal predicted value.

†Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in men (hematocrit, 55.5%) or >16.5 g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

Appendix 2: Transcript of the interviews with the different interveners of the hematological diagnostic

Clinician's point of view (interview with Professor G. Verhoef):

- In general, has the new WHO2016 classification changed/added much for your routine work?
  - Not really, no major changes. The biggest impact of the new WHO classification is for the acute leukemia because there is now a large amount of molecular markers available. This is still interesting for the clinicians but that essentially changed not so much. The message is, and always will be (and certainly for the acute leukemia) that cytogenetic and especially molecular markers define which entity it is and which type of treatment should be given.
    Another example (for the lymphoma): the molecular biology will become more and more important as the type of treatment would change related to the identified mutations.
    The new added or modified entities, from a clinical point of view, doesn't change the situation. For the myelodysplastic syndromes the new WHO 2016 classification does not change the routine work of a hematologist. The myelodysplastic syndromes still remain a frustrating category and the fact of a sub-classification into such or such entity does neither change a lot. For example, the new sub-category "MDS with ring sideroblasts and thrombocytosis" changes nothing in terms of treatment. Also, for the "MDS-EB-1 and MDS-EB-2", those new names make the thing more evident but changes nothing. Indeed, nowadays there are still not enough drugs which are efficient for this category. However, the molecular approach still remains important in terms of prognostic.
    For the myeloproliferative neoplasms, aside also some details, the major improvement is that the clinician can now pose the diagnostic "Polycythemia vera" or "Essential Thrombocythemia" sooner (with the lower diagnostic criteria).

- Which information (according to you and your implication into the hematological diagnostic process) are the most important to be found in a cytology protocol?

- The cytological diagnostic is still very important for the clinicians. As more accurate this diagnostic is, as more useful it is for the clinicians. It gives the clinician the opportunity to know what they must do (in terms of treatment).
- A differential diagnostic allows also a degree of uncertainty and it's useful. The chance of getting a final diagnosis already by the cytology step is also a good oppurtinity for the clinicians.
- The difference between MDS-EB1 and EB-2 is also meaningful. For a MDS-EB2 the prognostic is worse and can justify a stem cells transplantation.
- At the early stage of the MDS, the differential diagnostic with the reactive changes is still possible and not always easy.
- It is also a pity that the cytogenetics goes not always further. The cytogeneticists very often read the protocol of the cytologists and would go further as they see it actually is a MDS. If not, cytogenetics can be on hold.
  In a perfect and ideal world, it would be nice that cytogenetics would always be carried out (for example even with minimal dysplastic changes, because we could find cytogenetics abnormalities in those situations).

- What is your opinion to reach more standardization for our clinic? For example, what are the guidelines available for the clinicians (i.e. in terms of which or which complementary tests should be carried out)?
  - The literature obviously. Sometimes the situation is very clear. Most of the things are well known for the clinicians. Example: for the acute leukemia, the standard is: cytology, flow cytometry, molecular biology. The use of the Next-generation sequencing is not already crystallized. This technique is still a little bit experimental for diagnostic purpose.
    For the MDS is also evident that cytogenetics doesn't have to always be done.
- For MPN: I have observed that too often a flow cytometry is asked whereas is only useful in a limited indication such as a crisis of blasts for CML. How could we explain this?
  - The reason why a flow cytometry is often asked can be found in the fact that UZ Leuven is an university hospital and a place of formation for a lot of interns.
  - Also, there are patients who are included into clinical studies and then sometimes a flow cytometry of anything else could be asked even it is not always in accord with the guidelines.

- How can you measure the impact of the quality of the samples?
  - The examination of the blood smear is a great added value for the cytological diagnostic. Unfortunately, too frequently a blood smear is not prescribed in the same time as the bone marrow aspiration. An informatics tool should be implemented to oblige the clinician to accompany the bone marrow aspiration with a blood smear examination.
    For the quality of the samples, before the bone marrow aspiration were done by every intern in Internal Medicine which each needed to be properly trained. Now the focus is only for the interns in Hematology and in Laboratory Medicine.
    What helps the clinicians is also the given opportunity to examine themselves the bone marrow smears and realize if there is a technical problem in their way of pricking.
    The formation of the nurse who are present in the puncture room is also primordial. For example, it should be useful that they always say if there are medullary grains or not.

Cytologist's point of view (interview with Dr. C. Brusselmans)

- In general, has the new WHO 2016 classification changed/added much for your routine work?

    - This new classification has not so much changed the cytological criteria. Cytology is the first test in de diagnosis work-up and the cytological findings remain important. They are quickly available and therefore cytology can play the role of a dispatcher suggesting which other additional testing should take place.
      In the new WHO, cytogenetic and molecular features are more and more important. Our daily routine work teaches us that cytological findings still have a place for the final diagnosis (e.g. MPN/MDS: some mutations can be found in the different MPN/MDS without specificity and cytological findings can help for sub-categorization into MPN/MDS).
      The cytological appraisal of dysplasia is often subjective, hardly describable, requires a lot of expertise and have an impact on the final conclusion (blastosis/assessment of dysplasia, proposal of a differential diagnosis). We cannot forget that the WHO criteria are guidelines and that there are early-stage diseases which can be presented with pathognomonic findings but without every WHO diagnostic criteria (e.g. morphological features of CMML with an absolute monocytosis below the WHO criterion < 1.10^9/L).
      Cytology, as the other additional testing, is a part of the multidisciplinary work-up.
      N.B. The work of cytologist can also be challenging because the clinical information is not always available and these are important regarding the interpretation of morphological findings and also for classification (e.g. therapy-related neoplasms and/or secondary dysplastic changes).

- How can you measure the impact of the quality of the samples?
    - The quality of the sample is of great importance: on one hand for the cellularity assessment, on the other hand for appraisal of dysplasia (e.g. megakaryocytes) and blastosis. If the aspiration is less representative, the cytological findings can make less a contribution (e.g. assessing for ET while there are too few megakaryocytes on the bone marrow smears) and thus we have to strive for optimization of bone marrow aspiration (and also for biopsy).
      The peripheral blood examination is essential and should be performed altogether with bone marrow aspiration. It gives information about i.a. possible anisopoikilocytosis, dysplasia, blastosis and thus it is also integrated in the WHO classification.

- What is your opinion to reach more standardization for our clinic?
    - Standardization for cytology occurs with different means (SOP, interne formation, LAG Hemato-Atlas).
      More precisely, the MDHW ("MDHO) was implemented in May 2011 in LWS (LIS of UZ Leuven). It regroups into a unique and common report the following information:
        - clinical information
        - reports of cytology-pathology-flow cytometry-cytogenetic-molecular
        - conclusion of the multidisciplinary hematological staffs (MOC)
        - hematological classification of the disease
        - diagnostic probability index

      The fill-in method is mainly based on "Copy/Paste" of the reports into the specific fields in the LIS. This current method causes a work-overload. In this process, a lot of time and energy were put in on one hand for the implementation of the

MDHW by the Clinical Pathologist and the LIS support team and on the other hand for the manual introduction of the relevant clinical and diagnostic elements. The introduction of this data has to be a continuous process to create a database. Consequently, the hematological diagnose work-up requests should be multidisciplinary implemented in the LIS and also the introduction of standardized conclusions of the different additional testing in this common report is primordial to make the global hematologic process more efficient.

The standardized coding system is defined in the LIS and established in the MDHW report by summarizing the relevant elements.

There is also the possibility to integrate pictures in the LIS. Each patient is unique and can present diagnostic variations which have to be registered. For example:

- Illustration and comparison of the pathological and morphological pattern and confrontation with international guidelines and with data from literature.
- Monitoring of the report of morphological abnormalities both on individual and population levels.

Since May 2011, more than 3000 patient's files for bone marrow aspiration were integrated in our coding system. The analyze of this MDHW database provides a large diversity of data's i.e.:

- Distribution of diseases by age-group, unit, ...
- Diagnostic probability index by pathology
- Correlation and discrepancies between the clinical and diagnostic reports.

This large and detailed database asks for us and for the implicated hospital units to further investigate the potential bottlenecks which can occur by the hematological diagnosis. In this way, it was intended to optimize the diagnose in the daily clinical/diagnostic practice and to come to a better multidisciplinary integration.

Pathologist's point of view (interview with Professor T. Tousseyn):

- In general, has the new WHO2016 classification changed/added much for your routine work?
  - The modification that he, as pathologist, experiments is that there are more and more sub-categories which depends essentially on the molecular and cytogenetics' data. Therefore, a part of the diagnostic puzzle is fulfilled thanks to them.
    About the morphology criteria of the different diseases, there are no significant changes for the pathologists.
    For the diagnostic, the relevant elements are more and more found in the peripheral blood (by cytologists) and also with molecular or with karyotype testing.
    He has then the impression that the pathologist's role becomes more and more limited in the precise hematological classification.
    However, for the myelofibrosis, the pathologists can still have an important role more significantly as the bone marrow aspiration is a "dry tap". For the indications where the bone marrow architecture is involved, pathology can always give significant information.
    Thus, the classification into different sub-categories, according to him, depends more and more of the dysplastic features observed with the cytological preparations. The pathologists could not easily assess the dysplasia but more the general architecture of the bone marrow. However, they can objectify the dymegakaryopoiesis, the maturation pathways of the different lineages, the amount of blasts and the degree of fibrosis also.

- How can you measure the impact of the quality of the samples?
    - The bone marrow for the pathologist should be, according to the guidelines, of at least 2,0 cm long. In practice, it's almost never the case.
      This is tough important because the cellularity and the morphological abnormalities may locally vary. As the biopsy is not long enough, we could have what we call a sampling artefact. For example, a sub-cortical artefact is often the case. In the sub-cortical space, we have often 4 of 5 inter-trabecular spaces which are entirely empty (which is normal). But if the sampling is only taken into that specific region, the pathologist could have the false impression of an aplastic bone marrow.
      A good representativeness is crucial for this new WHO classification.

- What is your opinion to reach more standardization for our clinic?
    - The cytological rapport is only watched after the first examination of the pathology's smears to assess a good correlation between both the methods.
      He has the impression of for ¼ of the cases there is no correlation between cytology and pathology (e.g. non representativeness for the bone marrow aspiration in opposition to the biopsy).
      To reach more standardization, he would like to have the same informatics systems as used in the cytology laboratory.

<u>Cytogenetics & Molecular biologist's point of view (interview with Dr L. Michaux):</u>

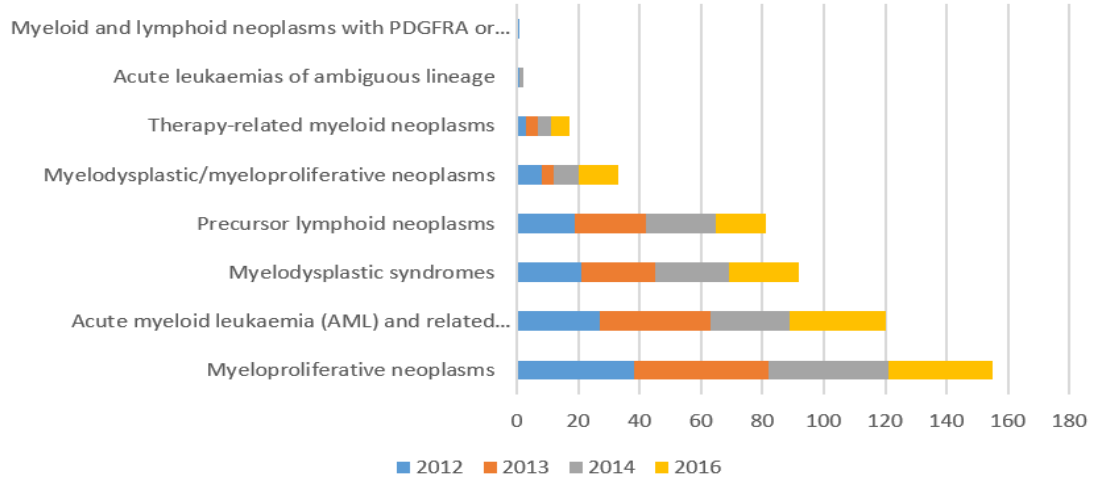
- In general, has the new WHO2016 classification changed/added much for your routine work?
    - For the cytogenetics and molecular work-up of the hematological diseases, there are no significant changes.
      Although, she has the impression that there are more and more insufficiently clinically justified prescriptions for Next-Generation Sequencing (especially for the extern: e.g. searching for PDGFR alpha/beta). It's also important to remind that NGS is currently not reimbursed in Belgium.
      We propose the use of Next-Generation Sequencing for hematological situations where there is sufficient data supporting its use. However, even in those situations, it is not always easy to explain the signification of particular mutations for our patients in terms of prognosis/diagnosis).
      Indeed, there are few multi-centric studies regarding the prognostic and diagnostic value of the newly discovered molecular features.

- How can you measure the impact of the quality of the samples?

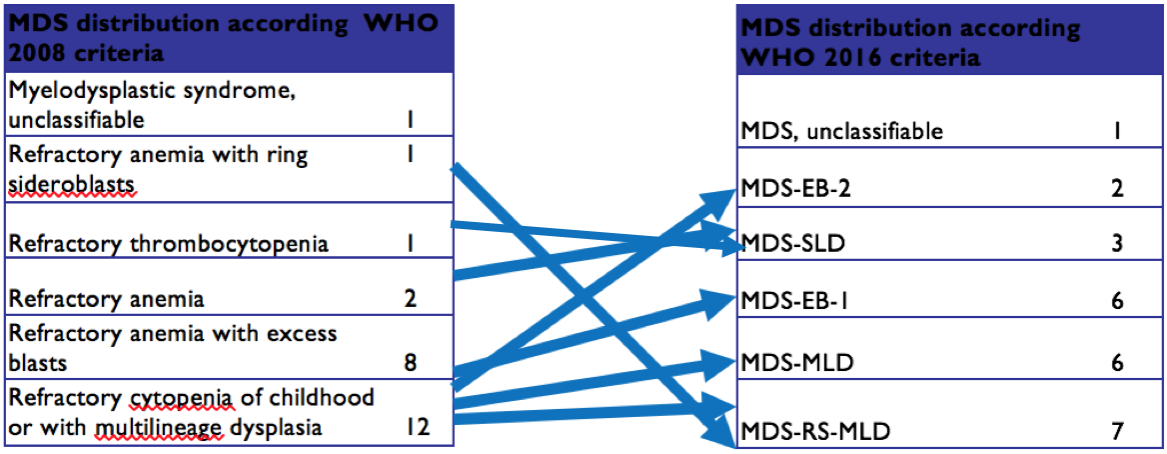
    - For cytogenetics, a normal karyotype requires the analysis of at least 20 mitoses. Unfortunately, that is not always achievable, due to several constraints.

- What is your opinion in an aim of reaching more standardization for our clinic?
    - The cytogenetics for myeloproliferative neoplasms should be performed preferentially on bone marrow (with the exception of primary myelofibrosis in which blood is preferred). The cytogenetic and molecular reports are completely standardized.

Appendix 3: incidence of MDS, MPN, MDS/MPN, acute leukemia patients for the year 2012, 2013, 2014 & 2016



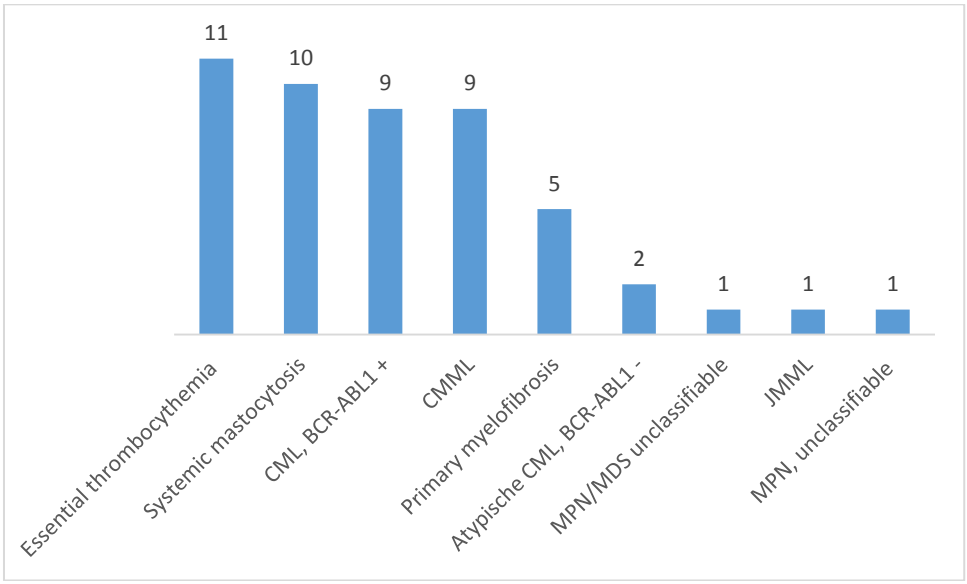
Appendix 4: Distribution of our cohort of MDS patients (n=25) according to the WHO 2008 criteria and to the WHO 2016 criteria



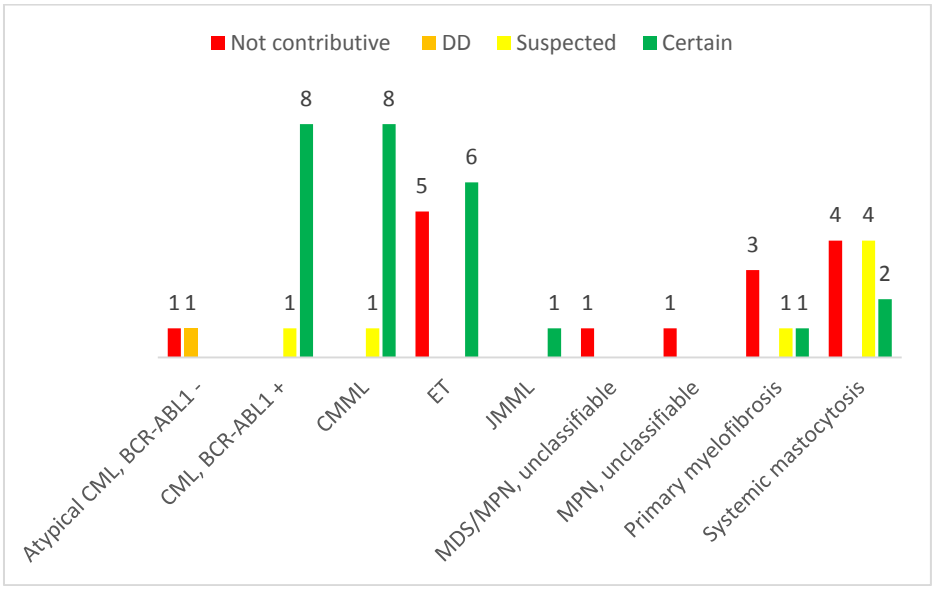
Appendix 5: Comparison of patient's distribution for our cohort MPN, MDS/MPN and Systemic mastocytosis between 2014-2016

Diagnosis	2014	2016
Atypical chronic myeloid leukemia, BCR-ABL1 -	0	2
Chronic myeloid leukemia, BCR-ABL1 +	8	9
Chronic myelomonocytic leukemia	6	9
Essential thrombocythemia	11	11
MDS/MPN, unclassifiable	2	1
Polycthemia vera	4	0
Primary myelofibrosis	8	5
Systemic mastocytosis	11	10

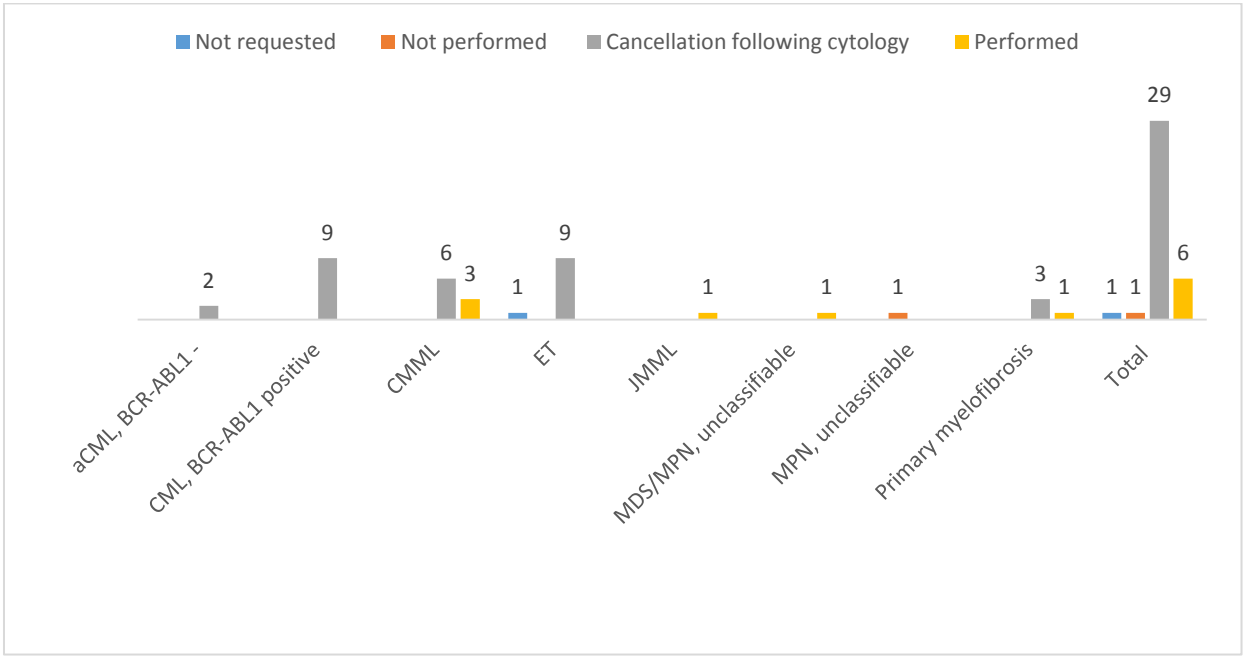
Appendix 6: Incidence of MPN, MPN/MDS and Systemic mastocytosis (UZLEUVEN 2016)



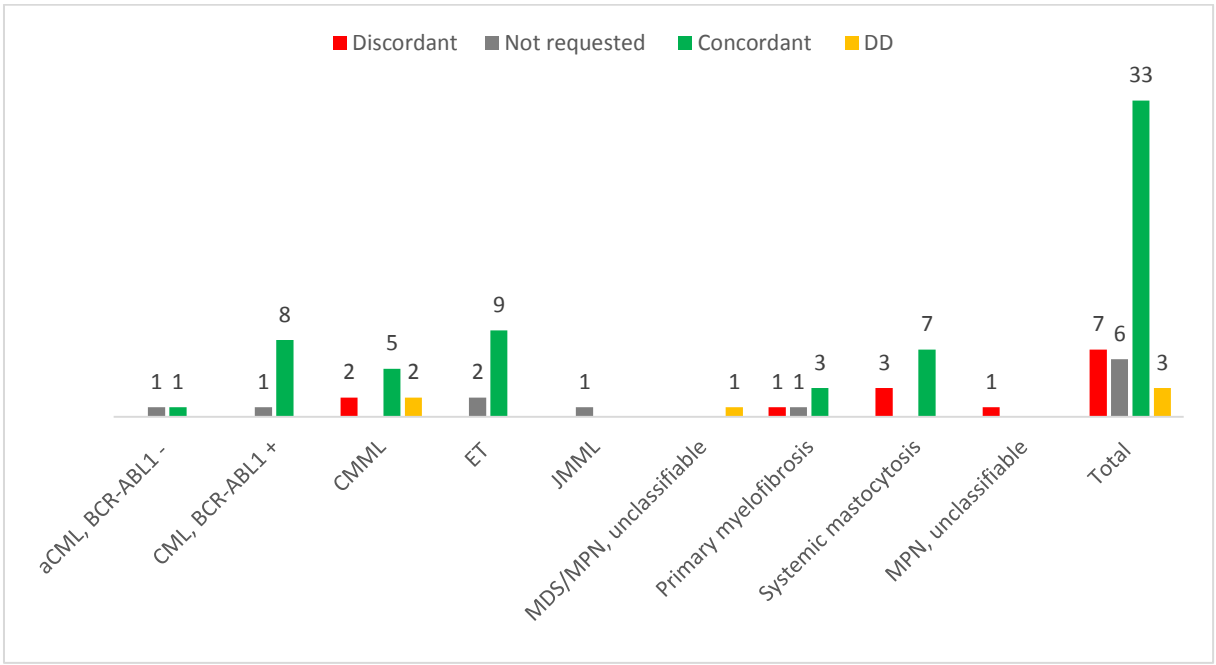
Appendix 7: Cytology contribution to the diagnosis for our cohort MPN, MDS/MPN & Systemic Mastocytosis



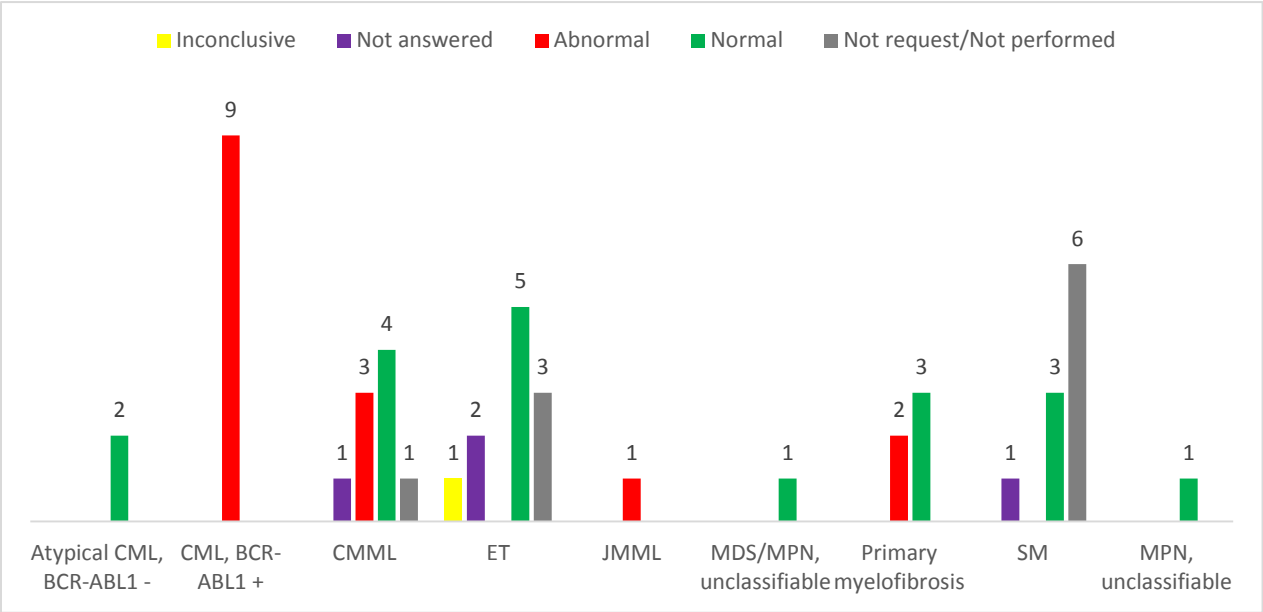
Appendix 8: Distribution of the flow cytometry requests into the MPN, MDS/MPN cohort



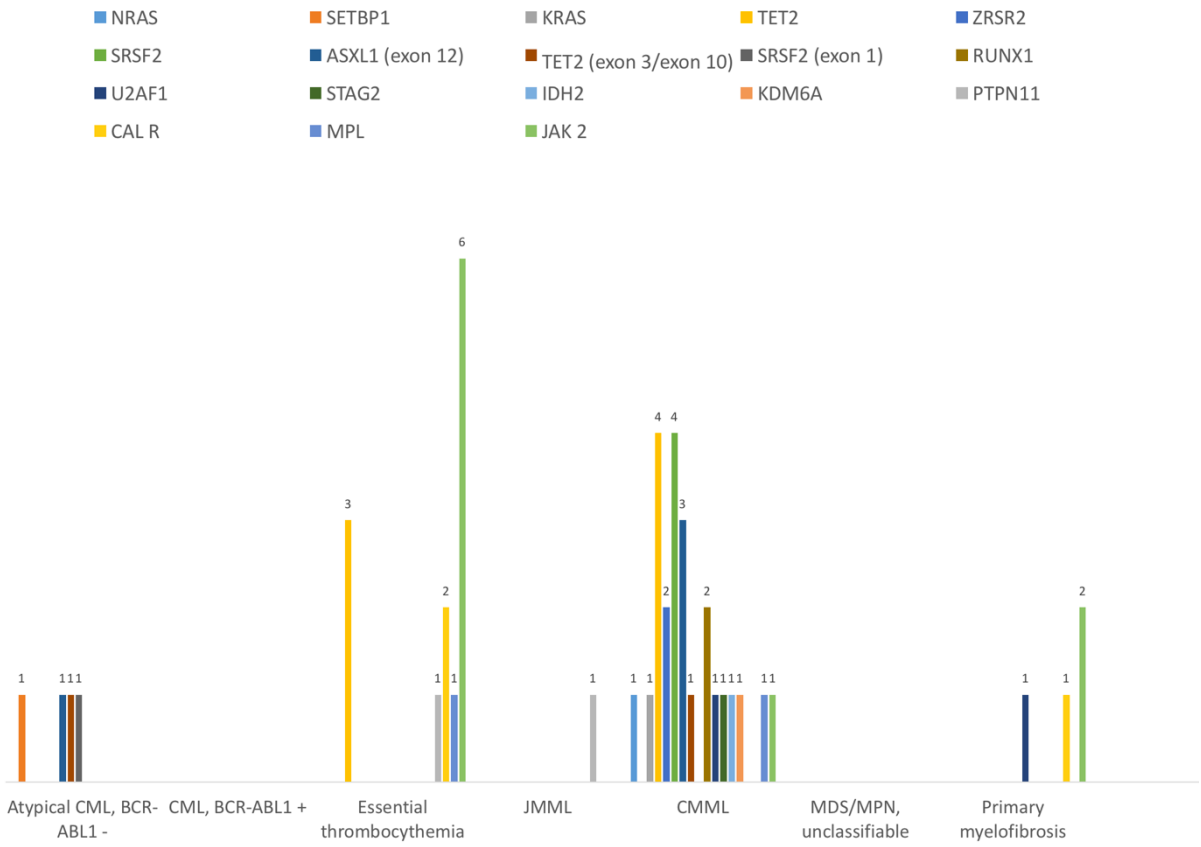
Appendix 9: Pathology contribution to the diagnosis for our cohort MPN, MDS/MPN & Systemic Mastocytosis



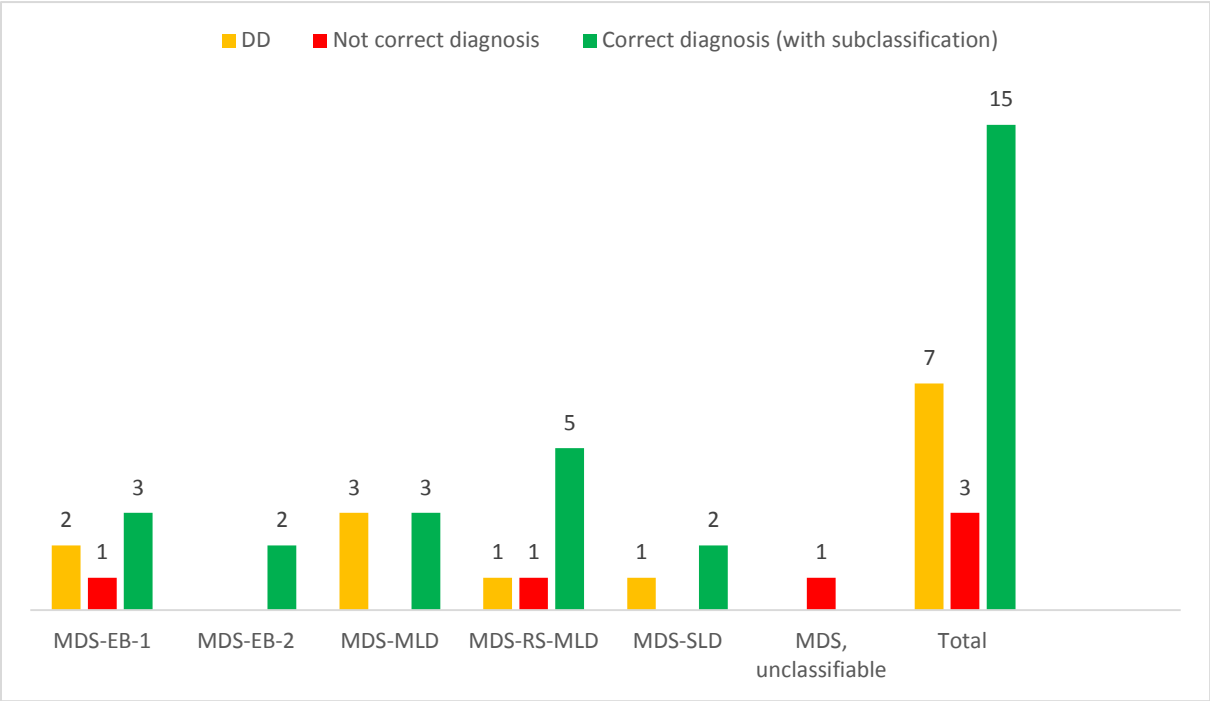
Appendix 10: Cytogenetic contribution to the diagnosis for our cohort MPN, MDS/MPN & Systemic Mastocytosis



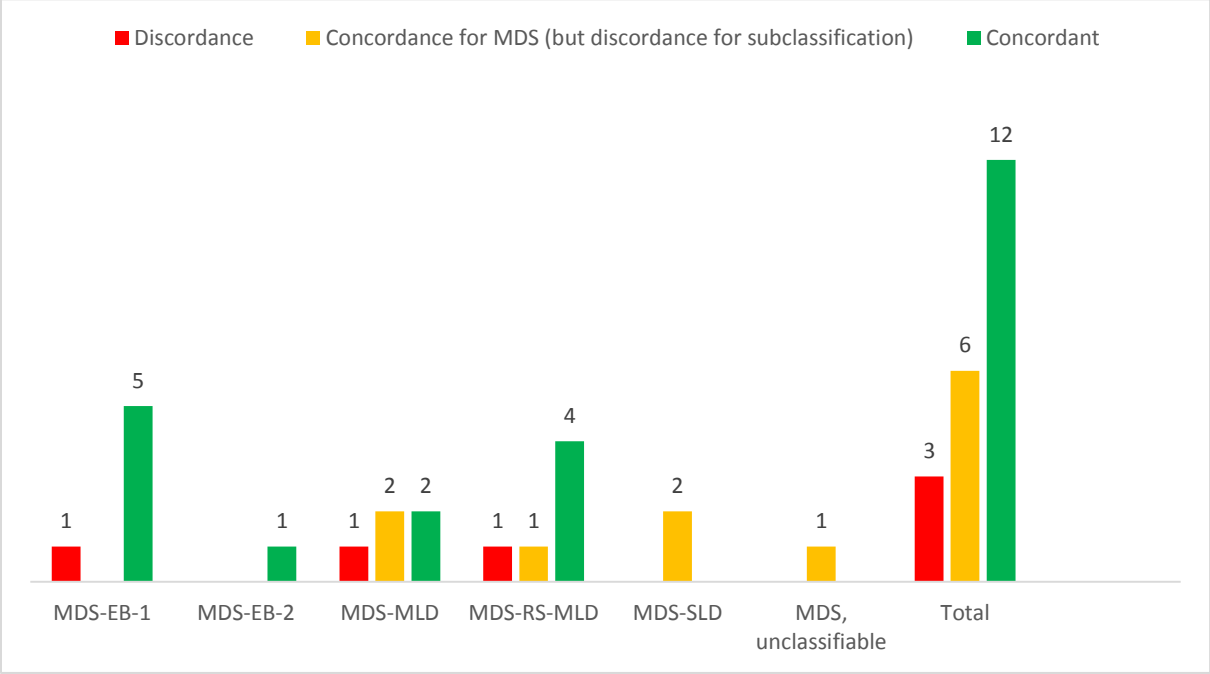
Appendix 11: Positive molecular markers for MPN, MDS/MPN by NGS



Appendix 12: Cytology's contribution to the diagnose for our cohort of MDS patients



Appendix 13: Pathology's contribution to the diagnose for our cohort of MDS patients



Appendix 14: Cytogenetic abnormalities distribution for our cohort of MDS patients

	Abnormal	Normal
MDS-EB-1	3 (complex karyotype (2), Pseudiploid karyotype (1))	2
MDS-EB-2	1 (complex karyotype)	1
MDS-MLD	3 (complex karyotype (1), loss of Y (1), del(20q) (1)	3
MDS-RS-MLD	2 (complex karyotype)	5
MDS-SLD		3
MDS, unclassifiable		1

***Appendix 15: Frequency of the mutated genes in our MDS patients' cohort compared to frequency described in literature* [2], [29], [4].**

GENES	LITERATURE	UZ LEUVEN MDS 2016
DNMT3A	12-18%	31%
TP53	8-12%	19.23%
SRSF2	10-15%	15.38%
TET2	20-25%	15.38%
SF3B1	20-30%	7.69%
EZH2	5-10%	11.54%
STAG2	5-10%	11.54%
IDH2	< 5%	7.69%
ZRSR2	5-10%	3.85%
JAK2	< 5%	3.85%
RUNX1	10-15%	3.85%
U2AF1	8-12%	3.85%
BCOR	< 5%	3.85%