

Chapter 10

General discussion and future perspectives

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Myelodysplastic syndromes is a heterogeneous group of clonal hematopoietic bone marrow disorders, characterized by cytopenias and increased risk of leukemic transformation. The diagnosis is established on the presence of specific cytomorphological (CM) findings, the presence of MDS-associated cytogenetic abnormalities or presence of a mutation in the splicing chromosome *SF3B1* (in the context of presence of ring sideroblasts).¹⁻³ In patients with cytopenia(s), distinction between clonal and non-clonal diseases can be challenging due to many reasons. On one hand, MDS cases might present with minimal dysplastic features, no increased percentage of blasts and normal cytogenetic analysis. On the other hand, dysplasia is not restricted to MDS, it is also seen in reactive conditions. Furthermore, cytogenetic abnormalities are absent in around 50% of the MDS cases and rarely MDS-specific.⁴ The diagnostic guidelines for MDS established by the European LeukemiaNet (ELN) recommend the addition of FC, and suggest additional molecular techniques to support the diagnosis of MDS.⁵ As described in the first two chapters FC is ready for clinical implementation in the diagnostic work-up in MDS, especially in excluding clonal disorders in patients with minimal dysplastic features and normal cytogenetics. The specificity (true negative) of MDS-FC analysis calculated after one year follow-up was 95%. This means that MDS-FC was very specific in excluding development of MDS within one year. The aim of the first part of this thesis was to evaluate and improve currently applied MDS-FC algorithms and investigate the efficacy of novel diagnostic tools in the diagnostic work-up in MDS.

This thesis provides recommendations for improvement of current applied MDS-FC panels. The first step was to review currently used MDS-FC approaches. Overall, the most commonly used diagnostic MDS-FC models have a sensitivity of 71% and specificity of 93%.^{6,7} Due to wide availability of CD-markers that describe the granulocytic differentiation and maturation, this cell compartment forms the cornerstone of FC algorithms.^{8,9} Our study focused on the differences between MDS and pathological controls, all patients with cytopenias. The most concise panel for proper distinction between these patient groups included: CD34, CD117, CD11b, CD13, CD16 and CD56. Most markers are already present in the proposal for AML/MDS analysis by the EuroFlow Consortium. In combination with CD56, this might provide a highly specific MDS-FC panel. The sensitivity of these parameters can be calculated after addition of the other cell compartments, as MDS is not diagnosed solely based on abnormal neutrophils. The lower sensitivity (around 71%) of most commonly used diagnostic MDS-FC models is partially caused by the absence of the erythroid cell compartment in these models. Therefore, we developed and validated the erythroid lineage evaluation and added it to applied MDS-FC algorithms. Only four parameters based on three markers (CD117, CD71, CD36) were sufficient to achieve an increased diagnostic sensitivity to 80%, without effecting the specificity (95%). To further increase the sensitivity from MDS-FC the megakaryocytic lineage should be added. Evaluation of the megakaryocytic lineage still faces technical challenges. However, the evaluation of CD34, CD36, CD42a and CD61 are very promising.¹⁰ Note that this means that addition of only 2 markers to the panel suggested above is sufficient. The megakaryocytic evaluation is currently under development within the ELNet MDS Flow Cytometry.

To keep, and improve the specificity, the same analysis as preformed for the granulocytes should be performed for the monocytes. There are multiple markers, that describe the monocytic cell population. The challenge here is to identify markers that distinct MDS from other clonal diseases such as chronic myelomonocytic leukemia and myeloproliferative disorders. Others suggest panels including CD14, CD16, HLA-DR, CD64, CD56.^{8,11}

Overall, evaluation of endless lists of CD-markers should be avoided. Shorter panels that evaluate the erythroid, myeloid progenitors, neutrophils, monocytes, and megakaryocytes, will lead to an overall higher specificity. FC can underline the diagnosis in indifferent cases, or even predict development of MDS in the near future. The role of other cell subsets such as dendritic cells (although very promising), and lymphocytes (except progenitor B cells) still need further investigation before introducing them in current standardized diagnostic panels, as their role in the identification of MDS is unclear. Aim should be to develop a standardized single tube multicolour FC analysis, which can be performed widely, and does not require high levels of expertise. Here new software can help to simplify the procedures. Panels need to be concise, easier to apply, less time consuming and cheaper.

Flow cytometry is described as an additional diagnostic tool to complement CM and cytogenetic analysis in suspected MDS. Note that, FC and CM evaluate different cell properties/aspects/features and therefore not necessarily correlate with one another. A big challenge in this thesis, and also in other studies regarding FC in MDS, is that FC results are always correlated to the gold standard. And here, the gold standard is CM features, in some cases supplemented by genetic abnormalities. Note that the goal of the current research is not to replace CM by other techniques but to complement or improve the diagnostic work-up. Because, as we demonstrate in Chapter 6, the current diagnostic subgroups described by the WHO classifications are very heterogeneous in many aspects (features described by the different tools). Therefore, we like to suggest that for future research of novel diagnostic tools such as FC, SNP-arrays, and next-generation sequencing, results should be correlated to other parameters such as clinical features (i.e. presence of cytopenias, auto-immune phenomena, etc.) or prognosis (overall survival, time until leukemic evolution, therapy response, etc.).

Other pitfall in MDS analysis is that MDS is a clonal disease, and during the course of the disease the clonal cell population modifies due to disease, patient or treatment influences. Here studies that compare early onset MDS patients (based on high specific MDS-FC models that predict clonal development) to high risk highly aberrant MDS patients are needed. Also long-term follow-up of patients is mandatory to gain more insights in disease development. Identification of type, function, and moment of occurrence of a certain mutation with or without abnormal phenotype will provide better models that can predict prognosis or even treatment response.

By exploring the data in chapter 6, we found correlations between specific FC aberrancies and mutational status irrespective to the WHO diagnosis. We found that mutations in epigenetic regulators and transcription factors lead to aberrant myeloid progenitors, granulocytes or monocytes according to FC. What is the clinical impact of these findings? Research on pathophysiological mechanisms behind these correlations is mandatory as it will provide new targets for therapy. As MDS is a clonal disease that changes over time, a single drug approach seems not sufficient. Techniques that can monitor clonal evolution might guide treatment discussions: if the clone starts to appear again, or starts changing a different therapeutic strategy is mandatory. Here FC has already proved its applicability in acute myeloid leukemia, but also other techniques such as next generation sequencing or mass cytometry are thinkable.¹² However, these are complicated often expensive tools that are not yet ready for general application. SNP-array might be a good alternative as it becomes more widely available by commercial platforms.

Treatment options in MDS include growth factors (erythropoietin or G-CSF), hypomethylating agents (decitabine or azacitidine), or immune-modulating drugs (lenalidomide). The only potent curative

option in MDS is an allogeneic hematopoietic stem cell transplantation (allo-SCT), upfront or after cytoreduction¹³. Although reduced intensity have decreased non-relapse mortality, overall survival remains around 30%. To improve allo-SCT outcome, more research needs to be performed in optimizing therapeutic strategies. As part of supportive care, most MDS patients receive multiple red blood cell transfusions (RBCT) during the course of their disease. Multiple transfusion can cause secondary hemochromatosis (reviewed in Chapter 7), a deleterious effect of iron accumulation in heavily transfused MDS patients. In the following chapters we evaluate the influence of RBCT on outcome in allo-SCT.

In chapter 8 we moved scenery to prognosis in high risk myelodysplastic syndromes (MDS) and treatment with allo-SCT. Due to high treatment-related morbidity and mortality and high relapse risk the current long-term overall survival rate is around 30%.¹⁴ Over the years reduced intensity regimens were introduced for the elderly and more frail patients to reduce non-relapse mortality. However, treatment selection and treatment timing remains challenging. Many retrospective analysis evaluated relevant prognostic parameters. In these studies MDS patients are often pre-treated, which leads to very heterogeneous patient cohorts in respect to patient and disease characteristics. To diminish these confounding factors the Chronic Malignancies Working Party of the EBMT analysed MDS patients treated by upfront allo-SCT, who were not extensively pre-treated. This part of the thesis aimed to identify prognostic parameters that aid treatment outcome prediction and assist patients selection for treatment with allo-SCT. The focus here, the influence of RBCT and its associated iron overload and iron toxicity. The retrospective analysis in chapter 9 formed the rationale for the prospective non-interventional study in chapter 10. The prospective analysis concluded that transfusion burden influenced progression free survival, without a significant effect on survival. Treatment outcomes after 36 months in this not intensively pre-treated patient cohort were 52% overall survival (95% CI: 45%-59%), 44% relapse-free survival (95% CI: 37%-51%), 26% non-relapse mortality (95% CI: 19%-32%) and 31% relapse incidence (95% CI: 24%-37%). Expected parameters such as age, blasts percentage and comorbidity had a significant influence on outcome after ALLO-SCT.¹³ As also recently described in another large patient cohort, regimen intensity had no impact on overall survival in this study.¹⁵ Outcome might be predicted by serum ferritin levels, irrespective to CRP levels. Administration of iron reduction prior therapy prior to allo-SCT had no impact on primary outcome, but iron reduction therapy during the first year after allo-SCT increased overall survival. This thesis suggested the reduction of transfusion related iron overload in the allo-SCT setting. The easiest way to achieve this, is to reduce transfusions amount by shortening interval between diagnosis and curative therapy. The prediction of time of diagnosis until to leukemic evolution is difficult by current prognostic models. Development of better prognostic models, will lead to better treatment decisions (type of therapy and timing of starting therapy).

In summary, the thesis illustrates the efficacy of conventional and novel diagnostic tools in MDS. We provided suggestions for improvement of currently implied MDS-FC algorithms with the suggestion of a minimal panel and by reducing number of granulocytic markers and by the addition of the erythroid lineage. We illustrated the disease heterogeneity within MDS with the application of different diagnostic tools. And provide suggestions to improve disease analysis in the future. The second part the thesis illustrated the deleterious effect of secondary hemochromatosis on long term survival in MDS patients treated with best supportive care and especially the negative impact in high risk MDS patients that undergo allogeneic ALLO-SCT. Here, a positive impact of iron reduction therapy was suggested.

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