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Bone marrow evaluation in patients with suspected aplastic anemia

Principal Investigator: Dr. M.R. de Groot

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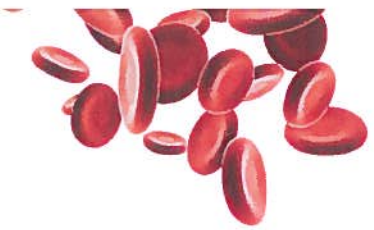
HEMATOLOGIE GRONINGEN



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SUMMARY

Rationale:

Rationale:

Acquired aplastic anemia (AA) is a hematopoietic stem cell (HSC) disease associated with bone marrow (BM) failure reflected by markedly reduced cellularity and deficient blood cell production. The pathophysiology of the disease is still not completely resolved; however aberrant activation of the immune system towards the HSC of the patient seems to play a central role. It is well known that after an inciting event, such as drug exposure or viral infection, the hematopoietic compartment can be destroyed by the immune system. Small numbers of surviving stem cells support adequate hematopoiesis for some time, but eventually the cell counts become very low and symptoms appear. The attaque on the bone marrow compartment might not only linked to hematopoietic stem cells but might also be directed to the surrounding microenvironment including mesenchymal stem cells (MSCs). MSCs play an important role in providing the specialized bone marrow microenvironment for hematopoietic stem cell survival and differentiation alterations and defects of these cells in AA have been described. Furthermore, co-transplantation of haploidentical mesenchymal stem cells to enhance engraftment of hematopoietic stem cells and to reduce the risk of graft failure in patients with failure has also been published supporting the role of MSC in AA.

In our laboratory we are able to characterize and culture MSCs. In order to study more extensively the possible alterations in MSC biology in AA we will investigate bone marrow cells of patients with newly diagnosed AA and at the time of response evaluation after immunosuppressive therapy.

Study design:

Observational study.

Study population:

Subjects with a suspected diagnosis of aplastic anemia at the age of 18 years or more will be asked to participate. Only those patients in whom the diagnosis is confirmed will be subjected to the second bone marrow biopsy. In those patients in whom the diagnosis is refuted the laboratory test described in this study will not be performed.

Main study parameters/endpoints:

Bone marrow mesenchymal stem cells of the patient will be collected and the interaction with the HSC cells will be analyzed in *in vitro* culture assays in conjunction with gene profiling.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

The sample for additional bone marrow will be collected during the standard diagnostic bone marrow punction and at the standard first response evaluation. In total 20 ml bone marrow cells will be collected at each punction. The collection of the sample will give a short stab during the suction procedure of the material from the bone marrow. The patients will experience no extra discomfort after the procedure.

1. INTRODUCTION AND RATIONALE

Acquired aplastic anemia (AA) is a hematopoietic stem cell (HSC) disease associated with bone marrow with markedly reduced cellularity and deficient blood cell production. The diagnosis is based on the presence depression of blood counts of at least 2 hematopoietic lineages and bone marrow hypocellularity (<30%).[1] The diagnosis of AA requires exclusion of systemic causes for marrow failure and nutritional deficiency. AA is rare in Western Europe and the United States (3-6 cases per million population per year). The pathophysiology of the disease is still not completely resolved, however aberrant activation of the immune system towards the HSC of the patient seem to play a central role.[2,3]Several immunosuppressive treatments have been developed in the past years to counteract this abnormal autoimmune attack ranging from drugs like cyclosporine, alemtuzumab and ATG.[4-6]. Depending on the severity of the disease at presentation, upfront allogeneic transplantation can be recommended as initial treatment, however treatment related toxicity and chronic graft vesus host disease have to be considered.[7,8]

It is well known that after an inciting event, such as drug exposure or viral infection, the hematopoietic compartment is destroyed by the immune system. Small numbers of surviving stem cells support adequate hematopoiesis for some time, but eventually the cell counts become very low and symptoms appear. Insufficiency of the hematopoietic microenvironment can also be an important factor. Mesenchymal stem cells (MSCs) play an important role in providing the specialized BM microenvironment for hematopoietic stem cells survival. Bone marrow MSCs from AA patients have the typical MSC immunophenotype, although aberrant cell morphology has been described, as well as proliferative defects, lower clonogenicity and increased cell apoptosis. Moreover the differentiation capacity of AA derived bone marrow MSCs appears defective.[9-15]

Furthermore, MSC have been used to improve the engraftment after identical allogeneic transplantation. Co-transplantation of haploidentical mesenchymal stem cells to enhance engraftment of hematopoietic stem cells and to reduce the risk of graft failure in patients with graft failure has also been published supporting the role of MSC in AA. [16-22]Thus,

MSCs are implicated in the development and treatment of AA and further study of these cells is warranted.

2. OBJECTIVES

The present study will be focused on defining the underlying differences between MSCs derived from patients with AA at presentation and MSCs derived from normal bone marrow. It will be defined in which degree the own microenvironment is supportive and behaves differently, and what processes are triggered by the altered micro-environment. MSC will be cultured from the CD34- cell fraction and after 2-3 passages MSCs can be used for studying the characteristics of the MSCs and the interaction with HSC. Subsequently it will be determined, how these changes translate in altered gene expression by performing gene profiling studies.
Furthermore, the BM sample at first response evaluation (3 months) will also be studied using the same study parameters in order to investigate whether the changes in MSC characteristics are reversible. In an exploratory analysis correlation between response on immunosuppressive therapy and MSC characteristics at presentation will be investigated. Bone marrow already obtained from healthy volunteers (a study of C Hazenberg investigating MSC biology in MDS patients and healthy volunteers) will be used as a reference.

3. STUDY DESIGN

In subjects diagnosed with AA additional bone marrow cells (20 ml) will be collected during the standard diagnostic bone marrow punction and again 20 ml at the first regularly (at 3 months) scheduled response evaluation.

4. STUDY POPULATION

4.1 Population (base)

- Subjects with a suspected diagnosis of aplastic anemia at the age of 18 years or more will be asked to participate. Only those patients in whom the diagnosis is confirmed will be subjected to the second bone marrow biopsy. In those patients in whom the diagnosis is refuted the laboratory test described in this study will not be performed.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- suspected diagnosis of AA
- ≥18 years

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Age < 18 years

4.4 Sample size calculation

Based on literature [9-14] it is estimated at least samples from 10 patients with confirmed diagnosis at the time of diagnosis and first follow-up have to be studied and compared with normal BM to obtain adequate information.

The number ot 10 relates to those patients with a confirmed diagnosis of AA. Thus, in case an included patient does not have the diagnosis he/she will be replaced. This will also be the case after withdrawal of consent.

5. METHODS

5.1 Study parameters/endpoints

Study parameters analyzed in collected bone marrow samples:

- MSC will be isolated from the CD34- cell fraction and after 2-3 passages MSCs can be used for studying the interaction with HSC in co-culture assays.
- Cultured MSC's will be studied for morphological changes, secretion of cytokines, cell surface markers and differentiation assays
- Subsequently it will be determined, how these changes translate in altered gene expression by performing gene profiling studies.
- Exploratory analysis correlation between response on immunosuppressive therapy and MSC characteristics at presentation

5.1.1 Main study parameter/endpoint

The functional characterization of MSC's in AA by gene expression profiling and in vitro behavior.

5.2 Study procedures

During the standard diagnostic bone marrow punction an additional 20 ml bone marrow sample will be collected. No additional puncture is required. The patient will experience a short stab during the suction procedure of the material from the bone marrow. MSC will be cultured from the CD34- cell fraction (and frozen) and after 2-3 passages MSCs can be used for studying the interaction with HSC via co-culture assays as well as other standard MSC laboratory studies already running in our lab. Subsequently it will be determined, how these changes translate in altered gene expression by performing gene profiling studies. In addition RNA, DNA will be isolated for defining the molecular markers. Response classification at the time of first follow-up will be scored according to the international response criteria.

5.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so, without any consequences.

5.3.1 Specific criteria for withdrawal (if applicable)

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

6. STATISTICAL ANALYSIS

The proposed *in vitro* experiments are explorative investigations and will generate new hypotheses. In line with the approach, the statistical analysis will be describing. This is also the case for the analysis of a possible relation between response and the observed MSC characteristics at diagnosis. For the latter the paired t test will be used. For the comparison of the continuous data between patients and controls a two sample t test will be used. For the non-parametric comparison of these two groups the Mann-Whitney test will be used.

6.1 Primary study parameter(s)

Parameters will be defined describing interaction of MSCs with HSC. These findings will be compared with results of similar experiments with MSCs from normal BM and from the same patient at first follow-up BM sample.

7. ETHICAL CONSIDERATIONS

7.1 Regulation statement

This study will be conducted in compliance with the International Conference on Harmonization (ICH) E6 Good Clinical Practice (GCP) guidance, and the most recent version of the Declaration of Helsinki as amended in 2013. In addition, all applicable local laws and regulatory requirements will be adhered to.

7.2 Recruitment and consent

The subjects will be informed and asked for participation by one of the staff members of the department of Hematology. Time to consider participation will be 7 days. The patient information letter and informed consent form are enclosed.

7.3 Benefits and risks assessment, group relatedness

The subject will not experience benefit from the tests performed. Hopefully, the treatment results of this patient group might improve as a result of these investigations in future.

7.4 Compensation for injury

The investigator has a liability insurance which is in accordance with the WMO regulation. No additional insurance is required since the investigation is not a threat for the subjects' health.

7.5 Handling and storage of data and documents

The material will be collected and frozen and stored with an anonymous code based on year data-sample number. In publications patients will be reported anonymously.

7.6 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

7.7 Annual progress report

The investigator will submit a summary of the progress of the investigations to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject and the total numbers of subjects that have been included. In addition a short summary will be given regarding the *in vitro* studies that have been performed.

7.8 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study.

7.9 Public disclosure and publication policy

The results of the scientific studies will be submitted to scientific journals.

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