K. Assessment of minimal residual disease

Minimal residual disease (MRD) detection in acute myeloid leukemia (AML) using PCR based techniques is applicable only in a minority of cases. Multiparameter flow cytometric MRD detection, using aberrant phenotypes defined at diagnosis, is applicable in roughly 80%-90% of the cases and has been shown to offer a strong prognostic factor independent of other prognostic factors in both adult and childhood AML (1-5). Both bone marrow (BM) after different courses of therapy (1-5), stem cell transplants (6) and 3 months after the last cycle of chemotherapy (7,8) have been applied for MRD assessment.

For the present protocol in all cases bone marrow samples and stem cell transplants have to be sent to the hematology department of VUmc. The methods for MRD detection are described in the SOPs present on [www.hematologie.nl/mrd/](http://www.hematologie.nl/mrd/)

**Definition of MRD:** malignant blasts as a percentage of the whole white blood cell compartment in complete remission material. These percentages are calculated based on the frequency of cells with an aberrant phenotype.

**References**

Bone marrow sampling:
1. At diagnosis*
2. After the 1\textsuperscript{st} cycle of chemotherapy \textbf{at day 30} after diagnosis (ie before the start of cycle II)
3. After the 2\textsuperscript{nd} cycle of chemotherapy prior to the start of cycle III or allo/auto SCT
4. In autologous stem cell transplants
5. Three months after the 3\textsuperscript{rd} cycle or high-dose chemotherapy/stem cell transplantation
6. At relapse (to recognize eventually occurring phenotypic shifts)

* Bone marrow samples of patients ≤60 years, who are suspected to have acute leukaemia, have to sent to VUmc (this is excluding the centers of Amsterdam, Rotterdam and Leuven, where a different approach has been settled).

\textit{Important}: this is prior to the time point at which the center has made the final diagnosis using morphology (and immunophenotyping). This thus is also prior to presentation of the patient to the HOVON data center for randomization. This strategy has to be followed since in most cases at the time point of randomisation the bone marrow sample is not appropriate for sending it to the VUmc (sample may arrive 2-4 days after aspiration).

Note 1: In few cases, already after sending the sample to VUmc, the diagnosis may turn out not be AML. In those cases the center should contact VUmc in order to prevent unnecessary further processing.

Note 2: For information only: after arrival of diagnosis material at VUmc, VUmc will do both the LAP establishment necessary for MRD detection and the purification of blasts by a ficoll centrifugation. The purified cells will be frozen en sent to the central laboratory at the Erasmus MC where the gene expression profiling will be performed.

Logistics:

- \textbf{Sampling conditions}: all samples should be obtained preferably from the first tap, gathered in heparin coated tubes and kept at room temperature. In any case it should be indicated:
  1. whether the sample is from the first or second tap
  2. when exactly (day and hour) aspiration take place

- \textbf{Volume of samples}:
  
  \textbf{At diagnosis and at relapse}: at least 5 ml BM.
  Int he improbable case, 10 ml of PB when blasts are present
  
  \textbf{After different cycles of chemotherapy}: at least 5 ml BM
  
  \textbf{In stem cell transplant}: 5 ml stem cell transplant

- \textbf{Announcement of a sample to VUmc}:
  The announcement of a forthcoming sample should be at least 1 day, preferably before 11:00 pm, prior to bone marrow aspiration. The expected time for a sample to be ready for transport and the place where it can be collected by the courier should be indicated on this announcement. The announcement should be done \textit{preferably} by phone.

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  Angele Kelder: 31204442624 or 31204443350
  Gerrit Jan Schuurhuis: 31204443838 or 31204442604 (coordinator of the MRD study):

  But also by E-mail:
  MRD@vumc.nl

This mail will arrive in the mailbox of the following persons: Nicole Feller, Angele Kelder, Guus Westra en Gerrit Jan Schuurhuis
If, for some reason (e.g. when a new patient presents with leukaemia), a forthcoming sample is announced on the same day (day 0) as the actual bone marrow aspiration, this should be done as early as possible in the morning, since hereafter VUmc has to inform the courier before 11.00 pm on day 0, in order to get the sample delivered at VUmc on day 1. When announced after 11.00 pm on day 0, the courier will collect it on day 1 and deliver it to VUmc on day 2, which is one day late. From this it follows that, whenever possible, we prefer to have bone marrow sampling on Thursday at last, since it can be delivered and processed at VUmc on Friday.

Please follow above mentioned procedure for announcing the sample since the actual MRD measurements should be done at last one day after the bone marrow collection.

- **Action of VUmc after the announcement of the center:**
  VUmc will contact the courier and provide these with the required information (location of the center and time of providing the sample). There are no financial actions for the participating center since all bills will be send to VUmc. The courier involved will be:
  “Special Delivery eXchange” (SDX) in Utrecht, The Netherlands.
  Tel: 31 302410106
  Fax: 31 2413311
  E-mail: john@sdx.nl
  Internet: www.sdx.nl

- **Sending of samples**
The samples have to be packed up (otherwise the courier is not allowed to transport it) and labelled with the address:
  VU medical Center
  Department of Hematology, PK 2 Br140
  To: N. Feller
  De Boelelaan 117
  1081 HV Amsterdam
  The Netherlands
  (Tel: +31204443836)
You will have to bring the sample to the arranged location (e.g. post office or porter) in the hospital, where it can easily be picked up by the courier for transport to VUmc.

For those who are interested: detailed procedures of MRD detection are on the website www.hematologie.nl/mrd/

For questions please contact: Nicole Feller (n.feller@vumc.nl) or Gerrit Jan Schuurhuis (gj.schuurhuis@vumc.nl)
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