Clinical implications of minimal residual disease monitoring by quantification of leukemia–specific mutations in patients with acute myeloid leukemia.

Louise Pettersson
Division of Oncology and Pathology, Department of Clinical Sciences, Lund
Main Supervisor: Mats Ehinger
Co-supervisors: Gunnar Juliussen, Lao Saal, Annica Pontén

Background
Optimal management of patients with acute myeloid leukemia (AML) depends on accurate monitoring of minimal/measurable residual disease (MRD), which predicts outcome and affects treatment decisions. Current MRD methods either suffer from limited sensitivity or can only be applied on leukemias carrying a specific targeted mutation. Multicolor flow cytometry (MFC) is the standard method, but may be unreliable.

Aim/Methods
In the first study, the aim was to compare MFC-MRD with RQ-PCR targeting the nucleophosmin 1 (NPM1) type A mutation in 15 AML patients including 45 follow-up MRD samples.

The aim of the second study was to investigate if relapses can be predicted by using an innovative digital droplet PCR (ddPCR) technique, IBSAFE, allowing targeting of multiple mutations. This offers the possibility to combine the knowledge of the mutational spectrum determined by next generation sequencing at diagnosis with the high sensitivity of IBSAFE, thus enabling monitoring of several putative subclones. The bone marrows from 10 relapsing and 4 non-relapsing AML patients were analyzed at diagnosis and at several follow-up time points.

Preliminary results
Study 1: In 32 of the 45 follow-up samples (71%), an MRD-signal could be detected with RQ-PCR targeting the NPM1 type A mutation. By contrast, only two samples (4%) showed residual leukemic cells as determined by MFC.

Study 2: For all relapsing patients, IBSAFE was able to track early recurrence of leukemic clones. At most follow-up time points, residual leukemia was apparent with IBSAFE, but absent with MFC-MRD. For the non-relapsing patients, some mutations were detected during follow-up, but the levels gradually declined in response to different therapeutic strategies.

Significance
RQ-PCR of the NPM1 type A mutation is more sensitive and reliable than MFC for determination of MRD. IBSAFE seems applicable on virtually all newly diagnosed adults with AML and clearly detects leukemic clones escaping MFC detection during follow-up. Both studies show that the detection of molecular MRD may be of importance for assessment of relapse risk with possible therapeutic implications.
Published paper

Pettersson, Louise; Levéen, Per; Axler, Olof; Dvorakova, Dana; Juliusson, Gunnar; Ehinger, Mats. Improved minimal residual disease detection by targeted quantitative polymerase chain reaction in Nucleophosmin 1 type a mutated acute myeloid leukemia. *Genes, Chromosomes and Cancer*, Vol. 55, No. 10, 01.10.2016, p. 750-766.