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COMMENTARY

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Can flow cytometry outperform genetic testing in eosinophilia patients?

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In this issue (page XXX) Sedigheh Sharifzadeh and her colleagues published a paper entitled "Phosflow assessment of PDGFRA phosphorylation state: A guide for tyrosine kinase inhibitor targeted therapy in hypereosinophilia patients." The authors investigated to what extent flow cytometry is applicable for phosphorylated molecules in eosinophil granulocytes and whether therapeutic consequences can be deduced for patients with hematological diseases, where clonal eosinophilia can be evaluated.

Platelet-derived growth factor (PDGF) binds to various forms of the PDGF receptor, most commonly consisting of alpha (PDGFRA) and beta (PDGFRB) chains.[1] This receptor has intracellular activating phosphorylation and binding sites for signaling molecules.[2] Mutations in these chains can result in an increased level of phosphorylation due to constitutively activated kinases.[1] Besides other mutations, this is one of the molecular mechanisms resulting in the clinical picture of clonal hypereosinophilia.[3]

Myeloid neoplasms with eosinophilia can be diagnosed according to standard guidelines. The methods are complex including the determination of white blood cell count, diagnostic imaging of different organs (e.g., hepato−/splenomegaly), serum markers (LDH, TnI/BNP, Tryptase, Vitamin B12, AP, IgE), bone marrow histology, detection or exclusion of BCR1-ABL1, FIP1L1-PDGFRA by RT-PCR or FISH analysis, ETV6-ABL1 by RT-PCR, performing of conventional cytogenetics from the bone marrow (4q12, 5q31-33, 8p11, 13q12) and confirmation of a fusion gene by FISH/RT-PCR as well as determination of phenotype mutations like KIT D816V, JAK2 V617F, STAT5B N642H, JAK2 ex13InDel and prognostic mutations such as ASXL1, SRSF2, RUNX1, EZH2, SETBP1, and others.[4] In addition, T-cell clonality by flow cytometric analysis or T-cell receptor rearrangement by PCR will be used.[4,5]

According to the WHO classification of neoplasms with eosinophilia, we distinguish myeloid or lymphoid neoplasia with eosinophilia and rearrangement of PDGFRA, PDGFRB, FGFR1, or with PCM1-JAK2 (MLN-Eo), chronic eosinophil leukemia, not otherwise specified (CEL, NOS), and idiopathic hypereosinophilic syndrome (HES).[5,6]

The measurement of phosphorylated transcription factors and receptor molecules is today well-established in flow cytometry. The method was initially only a research method.[7] In the meantime, applications in clinical diagnostics are increasing, but to the best of our knowledge no suitable in vitro diagnostic test kits for routinely determination of the phosphorylation state of PDGFRA by flow cytometry are available.

This is similar for most molecular genetic methods, and also in this case, the laboratories depend on their own expertise. Common applications in medical diagnostics are immunodeficiencies and immune function.[8–10] Furthermore, because STAT phosphorylation indicates activation of immunological cells, phosphorylation can also be used as a surrogate marker for antigen-specific activation in a time-saving manner.[8–10]

In brief, the laboratory applied a test kit for detection of intracellular phosphorylation and detected PDGFRA chain phosphorylation by a specific antibody. The results were compared with genetic testing. In addition, samples from other patients and cell lines were used as a comparison to ensure the specificity of the method. The level of phosphorylation was also confirmed via Western blot. The researchers' approach is noteworthy, given that the trend in diagnostics is rather more often applying genetic analyses than functional tests. In particular in oncology, detailed molecular biological investigations are now an essential basis for targeted individualized therapies.

However, the results certainly show also such a functional testing is useful. Not all patients exhibit the altered function of the PDGF receptor. Nine of 45 (20%) investigated patients with hypereosinophilia

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had higher levels of PDGFRA phosphorylation. In addition, in further investigations it becomes apparent that the genotype–phenotype linkage is incomplete also in this case: the authors detected PDGFRA-FIP1L1 fusion, ETV6-PDGFRA, and STRN-PDGFRA. This means that different mutations can be found with the test that cause increased mRNA expression.

This shows the possible particular relevance of this test: For therapy of such cases showing higher levels of PDGFRA phosphorylation, specific tyrosine kinase inhibitors can be used therapeutically and probably can act precisely on these phosphorylation sites. Thus, a prediction of those pharmacological effects could be expected. However, that must be further investigated in appropriate clinical trials with different kind of tyrosine kinase inhibitors. Since different mutations are associated with these diseases, a faster measuring of the functional changes of PDGFRA is probably a superior solution.

To what extent can such an examination be established in routine diagnostics? Medical laboratories must meet high quality standards (e.g., ISO 15189), and it is certainly not to be expected that an in vitro diagnostic test kit will come onto the market for such an assay.

Therefore, laboratories must be able to establish such tests themselves (so-called laboratory-developed tests) and perform them under quality-assured conditions. For the validation of tests in routine diagnostics, there are now updated recommendations that allow new tests to be introduced in compliance with guidelines and standards.[11]

Here, the authors show (in this issue page XXX) a well-developed protocol that can be established in laboratories experienced in flow cytometry. The volume of required blood sample is small, the processing is fast, reference intervals in a healthy reference population can be determined,[12] and strategies for quality assurance are available. The ROC curve shown suggests that the procedure developed is robust enough for a diagnostic decision that must be figured out in clinical trials.

The manuscript reports another example for methods of functional cytometry that no longer belong only to the research laboratory but are indispensable in routine diagnostics. It remains a challenge for laboratories to develop their own tests for routine diagnostics. However, the diagnostic information and the therapeutic consequences make it quite clear that the effort is definitely worthwhile.

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AUTHOR CONTRIBUTIONS

Ulrich Sack: Conceptualization; formal analysis; methodology; writing-original draft; writing-review and editing. **Stephan Fricke:** Conceptualization; formal analysis; methodology; writing-original draft; writing-review and editing.

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