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EDITORIAL

Molecular imaging and molecular diagnostics: two sides of the same coin?

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Back in the 1950s, it was a common concept to start further education in internal medicine with pathology [1]. The idea behind this was that a deeper knowledge of anatomy and pathohistomorphology of diseases would improve the accuracy of diagnosis and the success of treatment. It was during this period when internal medicine specialists started to utilize radioactive isotopes as tracers in biological assays, in vitro to enhance their understanding of molecular processes and in vivo to improve diagnostics – never losing sight of their ultimate goal of tailoring treatment to, and prolonging survival of, their patients. This period is today recognized as the birth of nuclear medicine.

The main drivers of nuclear medicine at that time (and still today) were the diagnosis and treatment of thyroid diseases. Based on a strong interdisciplinary background and education, it was self-evident that nuclear medicine diagnostics included not only scintigraphic evaluation of iodine metabolism, but also analysis of cytological findings. In addition, radioimmunoassays were developed for the analysis and interpretation of thyroid hormone constellations in the blood, a comprehensive theranostic approach which finally led to precise treatment with thyroid hormones and radioiodine. Given this historical development and the partly overlapping techniques applied, it is surprising that the obvious synergisms among nuclear medicine, pathology and laboratory medicine, which in many countries today is fused with clinical chemistry, have not yet led to greater collaboration among these disciplines on a broader level.

Today we are rapidly entering the age of personalized medicine, which means that molecular diagnostic techniques are increasingly used for the guidance of individualized therapy and the prediction of treatment response. As a consequence, the clinical field of pathology is today increasingly converting into a field of molecular pathology strategy. Driven by the recent evolution of technologies, including mass spectrometry and different "-omics" approaches, conventional pathology techniques such as immunohistochemistry are at present frequently accompanied by genetic, epigenetic and proteomic analyses which are available on-site in oncology centres and have become ever cheaper [2]. These technologies enable the identification of diagnostically and therapeutically relevant molecular targets and thus can be directly linked to modern biological treatments [3]. A review of the current literature verifies this concept in early clinical trials, in which a biomarker-driven rationale has been demonstrated to be superior to the conventional "one treatment fits all" concept.

However, while early clinical trials have proved the success of the new concepts, conflicting results and even failure of phase II and III clinical trials have been seen [4], and the overall likelihood of approval from phase I in oncological trials is still below 20% [5]. Today, the main limitation of these novel and more personalized concepts is widely recognized as the challenge of spatial and temporal tumour heterogeneity, where it has been shown that biopsy samples frequently underestimate the gene mutation burden of primary tumours, and that based on clonal evolution of cancer cells the biology of

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metastases can significantly differ from that of the primary tumour. It is the clonal evolution in particular that additionally drives spatial and temporal heterogeneity and resistance of tumours during treatment, so that the concept of guiding treatment from tissue samples is limited by nature [6]. Furthermore, with the introduction of hallmarks of cancer [7], the complexity of the relevant tumour supporting mechanisms has become clearer, in particular with the emerging hallmarks of tumour stroma interaction [8]. Given the plethora of new therapeutic agents on the market, we believe it is extremely important to understand drug reactions and tumour resistance, which, as stated above, are associated with three main areas: (1) cellular mechanisms, (2) tumour cell plasticity and (3) tumour microenvironment.

A promising approach to overcoming some of the limitations of conventional tumour biopsies for molecular analysis, especially with respect to cell autonomous mechanisms of tumorigenesis and to cellular plasticity, is the analysis of peripheral blood from cancer patients. In particular, molecular analysis of circulating cell-free (cf) DNA is on the verge of finding broad application in clinical practice. On the one hand, collection of cfDNA is by a standard blood draw from the patient and is thus minimally invasive compared to a conventional biopsy. This allows serial molecular analysis before, during and after treatment to monitor treatment response and clonal evolution of the tumour very closely to specifically address the temporal heterogeneity of the cancer. On the other hand, cfDNA represents the sum of the patient's tumour burden and not just a sample from one tumour location. This is of particular importance in metastatic disease where molecular analysis of a conventional biopsy from a single site often underestimates the heterogeneity of the tumour and might miss subclonal but clinically highly relevant mutations that confer resistance to targeted drugs. Thus, the use of cfDNA as a novel molecular tumour marker (often referred to as liquid biopsy) also has the potential to overcome the limitations of spatial tumour heterogeneity and to get more systemic information on tumour biology. This information can be further specified by adding bioinformatic analyses to estimate the probability of certain target expression or to simulate the course of the disease [9]. A recent trial in more than 1,000 patients with different types of cancer of stage I to III and 812 patients without cancer serving as controls has demonstrated that the specificity of these tests is very high (only 7 of 812 individuals without cancer showed positive results). However, depending on the tumour type the detection rates ranged between 30% and 95%. Particularly in early stage tumours, the detection rate of about 40% was only limited [10].

Still, from a molecular imaging point of view, we would prefer to "see what we are treating". It is obvious to molecular imaging experts that based on the possibility for whole-body imaging, multiple lesions and different tumour regions can be covered in real time, serially and noninvasively. Given the availability of a huge number of radiopharmaceuticals, it is possible to visualize and quantify specific targets in vivo, which also improves the understanding of tumour biology in vivo. It is also thus obvious to use terms such as "in vivo pathology" or "virtual biopsy" for these techniques. There are a number of studies that have nicely proved this concept, where the visualization and quantification of oestrogen or Her2/neu receptors in breast cancer [11, 12] or EGF receptors in patients with non-small cell lung cancer [13, 14] were directly linked to specific biological treatment. In the field of nuclear medicine these theranostic approaches are regularly applied not only in the field of thyroid diseases, but also in neuroendocrine tumours [15] and prostate cancer [16].

Molecular analysis of tumour tissue cfDNA and molecular imaging are complementary techniques with specific advantages and disadvantages. Testing tumour tissue allows both very sensitive detection of genetic aberrations within the specimen and the collection of topographic information on the tumour and the microenvironment at the cellular level. The importance and advantage of single cell resolution in histopathological analyses in comparison with molecular imaging relies especially on the possibility of detecting the interaction between tumour and stromal cells, which is of paramount importance for immunotherapies. However, these results are limited to the site of biopsy and are very likely not representative of the tumour mass in its entirety, and this is particularly the case in metastatic disease. In contrast, molecular imaging can visualize pathogenic processes at different tumour sites throughout the whole body simultaneously, but does not achieve resolution at the cellular level. Analysis of cfDNA has also been demonstrated to be very sensitive, but provides integrated information from all tumour sites rather than any topographic information. An additional advantage of cfDNA testing is the high temporal resolution with serial sampling throughout the course of the disease that cannot be achieved with any of the other techniques.

Therefore, a combination of all these approaches is needed for optimal management and monitoring of the patient at diagnosis, during treatment, in remission and at relapse. Several studies have already demonstrated the benefit of merging information from biomarker analyses of blood samples with molecular imaging. It is, for example, well known that the rate of detection of biochemical recurrence of prostate cancer by ^{68}Ga-PSMA PET increases with the amount of PSA tumour marker detected in the blood [17]. It has also been shown that combining tumour markers with [^{18}F]FDG PET in the differentiation of benign from malignant lung lesions is superior to the use of [^{18}F]FDG PET alone [18]. Initial studies are also demonstrating that the metabolic tumour burden significantly correlates with tumour cfDNA in patients with non-small cell lung cancer. Both tumour lesion glycolysis and mutations in cfDNA have been identified as independent predictors of event-free survival, which opens up opportunities for

complementary use particularly in the setting of patient follow-up [19, 20].

Although the involvement of specialists in pathology, laboratory medicine, medical genetics and nuclear medicine with their different areas of expertise is essential for optimization of the performance of the respective assays and profound interpretation of the results, it remains challenging to combine all the information to give a meaningful report for a specific clinical issue in an individual patient. Interdisciplinary cooperation of different specialists in molecular diagnostics is already required today in many cases and will become a *condicio sine qua non* in the future. An ideal scenario, particularly for treatment guidance in the context of targeted therapy, would be close cooperation and integration of pathology, laboratory medicine, medical genetics, nuclear medicine and potentially also other diagnostic specialties in one diagnostic centre. Such an integrated molecular diagnostic approach is clearly more than just the sum of its parts. On the one hand, it guarantees a comprehensive report for the physician (which could also be a nuclear medicine physician) treating the patient, instead of individual and potentially even conflicting results from single tests that can barely be interpreted without a profound knowledge of the specific possibilities and limitations of the respective analyses. On the other hand, such a setting may help choose the ideal combination of diagnostic tests for the clarification of a specific clinical question from a sometimes overwhelming repertoire – thus preventing overdiagnosis and underdiagnosis. Therefore, such a close cooperation and integration might be cost effective solely in terms of guiding diagnostic procedures. In addition, there are a number of synergies among the disciplines. For instance, molecular analysis of DNA isolated from tumour tissue or cfDNA requires similar technical infrastructure, wet-lab processes and bioinformatics pipelines – arguing strongly in favour of a close cooperation between pathology and laboratory medicine. Likewise, antibodies, minibodies, small molecules and molecular tracers can be developed, optimized and crossvalidated in parallel on different scales of resolution (from subcellular and cellular up to whole-body imaging) in classical in vitro diagnostic disciplines and in nuclear medicine.

The border between in vitro and in vivo diagnostics has begun to collapse. Novel technologies allow these different techniques to be combined to provide a comprehensive and (partly) noninvasive molecular analysis in the diagnostic work-up of cancer patients. This offers great opportunities to develop novel and better diagnostic algorithms to give the clinical physician the optimal tool to choose the right treatment from a rapidly increasing repertoire of (targeted) drugs and to guide the management of the patient. These algorithms can further optimize diagnostic solutions by application of machine learning and AI. In this way, refined in vitro and in vivo diagnostics represent the basis of personalized medicine. To be prepared for this challenging task, we believe that it is necessary to establish and deepen close cooperation and integration of different medical specialties to create a highly interdisciplinary setting.

Prof. Dr. Rudolf Höfer, one of the pioneers of nuclear medicine in Europe and a former director of the Department of Nuclear Medicine in Vienna, Austria, stated in 1995: “The organization of nuclear medicine is not a thing that is. It certainly was only yesterday a very different affair and, whether we assert control or leave everything to drift, nuclear medicine will be something different tomorrow” [21]. Given the rapid development in molecular diagnostic und the undoubted demand to improve the prediction of treatment response and outcome, both nuclear medicine and classical in vitro molecular diagnostic disciplines should start to be redefined to position nuclear medicine for tomorrow.

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