Diagnostics using cell-free circulating DNA from the blood: On the way into routine diagnostics?

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"Cell-free circulating DNA" (abbreviation: ccfDNA) from blood plasma or serum is becoming a more and more important building block of molecular diagnostics. It is seen as a way of replacing or complementing biopsies made through costly and onerous surgical interventions with simple blood collections, that is, non-invasive collection of diagnostic samples. I would therefore first like to explain the background on this new diagnostic tool, and then address some decisive aspects for its practical use.

Since the middle of the previous century, it has been known that free DNA – that is, DNA not bound to cells – is generally found in blood plasma, even if only in very small quantities of a few nanogrammes per millilitre. Even the knowledge that the amount of this DNA in the plasma may rise strongly in some diseases – particularly in specific tumours – had already originated in the 1960s and 1970s. However, it wasn't until the 1990s before researchers recognised that this DNA can be used as a tool for molecular diagnostics. The detection of tumour-specific mutations in this DNA was ground-breaking; furthermore, it was discovered that the DNA of the foetus is also found in the circulating DNA in pregnant women[1].

This foetal DNA is the basis for the nowadays often used non-invasive prenatal diagnostics which are commonly abbreviated as NIPT ("non-invasive prenatal testing"). Herein, "non-invasive" is to be understood as contrary to the previous standard method of amniocentesis (testing of the amniotic fluid), which is associated with a risk to the foetus that must not be underestimated[2,3]. In NIPT, a highly sensitive procedure (in most cases, a high-resolution sequencing technique called "Next Generation Sequencing" [NGS], is used here) is applied to allocate the foetal DNA segments which were obtained from the plasma to the various chromosomes. Since the expected number of these pieces is known with a normal chromosome count, a corresponding anomaly can be assumed when these figures deviate. This enables early detection of trisomies of chromosomes 21, 13 and 18 or Turner syndrome, wherein female foetuses have only one instead of two X chromosomes. In German-speaking regions, these diagnostics are very reliably performed by several providers within 1 2 weeks (e.g. PraenaTest®, HarmonyTest® or Panorama Test®).

The diagnosis and therapy support of tumour disorders is another field in which the use of circulating DNA is becoming more and more important. As mentioned above, it has long been known that dead and dying tumour cells release fragmented DNA into the bloodstream. This occurs primarily via two processes: first, apoptosis, directed cell death; and second, necrotic – that is, undirected – death processes of the tumour tissues. Apoptosis constantly takes place in all tissues, while necrotic processes are triggered primarily by applicable therapies using chemotherapy agents or radiotherapy[4]. Various clinical statements can be derived using the tumour cell DNA which was isolated from the plasma and the tumour specific information which is encoded on it: Early detection programmes can be used to detect tumours even in very early stages, which significantly improves the chances of being cured. This is already possible e.g. for intestinal cancer, using commercial tests (e.g. Epi proColon®). The success of a treatment method can also be quantified more simply in terms of therapy support than, for example, using imaging procedures. It is also of the utmost importance that a relapse of the disease can be determined very early (e.g. Colvera™). The analysis of the mutations found in the circulating tumour DNA can also be used to assess the efficacy of some medications in advance, and thereby avoid potentially useless treatments.

Aside from these two applications of cell-free circulating DNA which are currently the most important, there are other fields in which their use appears imminent, e.g. in organ disorders such as diabetes, or in supporting patients who have received organ transplants.

As described, cell-free circulating DNA can be a very helpful analyte in a variety of fields. However, some technical conditions are decisive for this; particularly those associated with the collection, transport and storage of the samples, and processing of the DNA. These work steps before the actual test are also referred to as pre-analytics. Due to the importance of pre- analytics, the EU defined a corresponding technical laboratory standard for diagnostics based on cell-free circulating DNA[5].

Cell-free circulating DNA is only found in the plasma in very small quantities. In order to make it possible to detect this target DNA using the highly sensitive analysis methods, it is essential that it is not overlaid or diluted by DNA from the blood cells. The blood cell DNA does not contain the disease-relevant information and rapidly makes it impossible to determine the chromosomal frequency. Even directly after blood collection, e. g. into a standard tube with an ▸▸

Continued... Diagnostics using cell-free circulating DNA from the blood: Soon to be part of routine diagnostics?

anticoagulant (EDTA tubes), white blood cells begin to die off and release their DNA into the plasma. This process is further accelerated when the blood samples must be stored at room temperature or higher temperatures before further processing, or transported to an analysis laboratory. Red blood cells also begin to lyse over time. These do not contain DNA; however, the increasing red colouration of the plasma makes it increasingly difficult to separate the plasma from the cells. Also, all cells swell noticeably over time. This greatly reduces the plasma yield. Since the target DNA molecules are only present in very small quantities, large amounts of cell-free plasma are required for many tests.

In order to counteract these processes, various manufacturers have developed blood collection tubes which stabilise the blood cells and thereby prevent them from releasing their DNA into the plasma. These tubes make it possible to store and transport blood samples at room temperature and higher temperatures for several days. This effect is frequently obtained by placing a reagent in the tubes which links biomolecules with each other, resulting in so called "cross-links"[6]. This prevents the cell membranes of the blood cells from bursting and releasing the DNA from the cells into the plasma. However, these reagents also link other biomolecules such as the target DNA, which causes problems in some analytical tests. These disadvantages were avoided in the development of the new PAXgene® Blood ccfDNA system by using a different technology which does not use linking reagents, and very effectively stabilises both white and red blood cells (Fig. 1).

On the one hand, this system consists of a blood collection tube which – unlike other tubes – is made from unbreakable plastic and has a proven spray shield (BD Hemogard™) in the closure to avoid contamination of the surroundings by blood. On the other hand, the system includes a precisely matched process which was developed specifically for this tube for efficient cleaning of the cell-free, circulating DNA. Since this procedure is performed by a laboratory automaton, human errors are minimised, and more samples can be processed. Due to this high level of standardisation, it is ensured that there are no losses of the valuable target DNA due to pre-analytical errors which might negatively influence the test results.

In summary, it can be clearly stated that the analysis of cell-free circulating DNA will become more and more important in the future, far beyond the currently existing applications in diagnostics. A benefit for science is seen here for more and more new disease fields, and the technical prerequisites for broader application in clinical practice already exist.

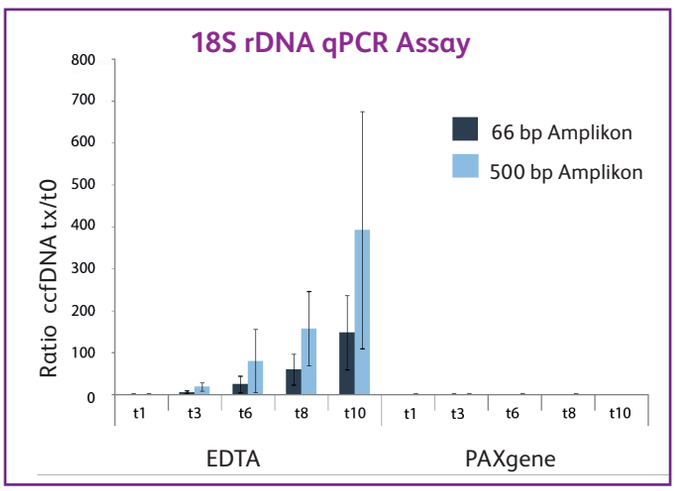

Fig. 1: PAXgene Blood ccfDNA stabilisation prevents the release of genomic DNA from white blood cells into the plasma. Plasma from six donors was separated from the blood cells at various times; the ccfDNA was isolated and its quantity determined using "real-time PCR" (18S rDNA gene, 66bp/500 bp amplicon). t = days, t0 = time directly after blood collection tx = time period of storage at room temperature. EDTA: conventional blood collection tube, PAXgene: PAXgene Blood ccfDNA blood collection tube.

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