Commentary

The struggle to detect circulating DNA

Sacha Zeerleder

Sanquin Research at CLB, Department of Immunopathology, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands

Corresponding author: Sacha Zeerleder, s.zeerleder@sanquin.nl

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See related research by Rhodes *et al*., http:ccforum.com/content/10/2/R60

Abstract

In various diseases, such as cancer, autoimmune disease, sepsis or myocardial infarction, elevated levels of circulating DNA can be measured. However, its predictive value is under debate. Circulating DNA in plasma is protein-bound (nucleosomal) DNA. Quantification of circulating DNA can be performed by real-time quantitative PCR or immunological methods such as ELISA. The diagnostic value of both methods can be impaired by inappropriate handling of the samples. Assessment of circulating DNA in patients admitted to the intensive care unit offers a tool for predicting morbidity and mortality.

Introduction

In the previous issue of *Critical Care*, Rhodes and colleagues [1] report on significantly increased levels of circulating DNA in patients admitted to the intensive care unit (ICU) in comparison with healthy controls. They show plasma DNA levels to be an independent predictor of mortality and the development of sepsis in these patients. In sepsis and trauma, circulating nucleosomal DNA is positively correlated with disease severity and adverse outcome [2,3]. In cancer, changes in circulating DNA levels have a prognostic value [4]. Interestingly, in systemic lupus erythematosus, an autoimmune disease in which nucleosomal DNA functions as autoantigenic target, no correlation of circulating nucleosomal DNA with disease severity can be found; instead there is a correlation with anti-nucleosomal DNA antibodies [5]. It is very likely that these antibodies take care of enhanced clearance of nucleosomes.

Circulating DNA

DNA in plasma most probably circulates bound to proteins in the form of mononucleosomes and/or oligonucleosomes and is released after the cleavage of easily accessible linkage sites of cellular DNA by endonucleases after cell death [6]. A mononucleosome consists of a core particle composed of an octamer of two copies each of histones H2A, H2B, H3 and H4, around which a stretch of helical DNA 146 base pairs in length is wrapped. Oligonucleosomes are composed of variable amounts of mononucleosomes connected by intact linker DNA with a variable length of 15 to 100 base pairs containing a 'linker' histone H1. Once released into the circulation, nucleosomes seem to be protected by their structure from further degradation by endonucleases [7].

In healthy individuals, the concentration of circulating DNA is low, because dead cells are removed efficiently from circulation by phagocytes. Circulating DNA has a short half-life (10 to 15 minutes) and is removed mainly by the liver [8,9]. Accumulation of DNA in the circulation can result from an excessive release of DNA caused by massive cell death, inefficient removal of the dead cells or a combination of both. Rhodes and colleagues demonstrate that increased circulating DNA not only predicts the development of sepsis but also mortality in patients admitted to the ICU [1]. Moreover, they show that patients requiring renal support have significantly higher values of circulating DNA than patients with sufficient renal function. Unfortunately, the authors provide no information on liver function, because most nucleosomal DNA is efficiently cleared by the liver and only a small fraction is eliminated by the kidney [8,9]. Our recent study in patients with sepsis showed that nucleosomal DNA increased with disease severity, but we found no difference in nucleosome levels in patients with severe renal insufficiency and normal renal function, respectively [2]. Other studies in patients with trauma and stroke showed that increased circulating DNA levels were correlated with morbidity and mortality [3,10]. Hence, assessment of circulating DNA offers a useful tool for predicting mortality and morbidity of patients admitted to the ICU. Further studies on circulating DNA in ICU patients, including more patients and other scoring systems for illness severity such as SAPS II

ELISA = enzyme-linked immunosorbent assay; ICU = intensive care unit; LOD = logistic organ dysfunction; RQ-PCR = real-time quantitative polymerase chain reaction.

(Simplified Acute Physiology Score II), logistic organ dysfunction and APACHE II (Acute Physiology and Chronic Health Evaluation II) scores, are needed to establish circulating DNA as a predictor for mortality and morbidity in patients admitted to the ICU.

Assessment of circulating DNA: real-time PCR versus immunological methods

Quantification of circulating nucleosomes can be assessed either by real-time quantitative PCR (RQ-PCR) or immunological assays. The RQ-PCR technique is widely accepted to quantify circulating DNA in plasma. However, contamination of a sample with nucleated cells can affect the apparent concentration of circulating DNA. Sample preparation to separate plasma from cells is therefore crucial. Chiu and colleagues [11] showed that a two-step procedure of sample centrifugation (800*g* or 1,600*g*) followed by either high-speed centrifugation or filtration was superior to a single centrifugation step only. Nevertheless, a 13.5-fold variation in circulating DNA levels over 3 days can be detected in female volunteers [12]. Therefore, even though an appropriate sample preparation protocol may be used, notable variation requires a careful interpretation of circulating DNA levels [12].

Nucleosomal DNA can also be assessed by ELISA technique as recently described by different groups [13,14]. In our laboratory we developed an ELISA with the use of a mouse monoclonal anti-histone 3 antibody (CLB-ANA/60) as a catching antibody and a monoclonal mouse antibody recognizing an epitope exposed on complexes of histone H2A, histone H2B and double-stranded DNA, present only on nucleosomes, as a detection antibody [14]. This technique renders quantitative determinations reliable and reproducible [2,13,14]. Also with ELISA, careless blood withdrawal and delayed centrifugation can result in false positive results, and insufficient storage conditions can lead to false negative results [13]. Moreover, sandwich ELISAs are vulnerable to false positive results resulting from xenoantibodies, C1q, rheumatoid factors and anti-nucleosome antibodies (L Aarden, unpublished work).

A comparison of RQ-PCR and ELISA methods revealed a high concordance in the quantification of circulating DNA in plasma and serum [15]. Both methods therefore have applications in measuring circulating DNA. Quantification should preferably be performed in plasma because, probably as a result of the clotting process, higher levels of circulating DNA can be measured [16]. However, determination of circulating DNA by RQ-PCR seems to be more sensitive than by ELISA. The lowest circulating DNA level measured by RQ-PCR in the present study was 14 ng/ml, which corresponds to 2,121 genome-equivalents/ml (assuming a DNA content of 6.6 pg per cell) [1]. However, circulating DNA can be detected by RQ-PCR up to 2 genome-equivalents/ml. In our recent study on nucleosome levels assessed by ELISA, we reported a detection limit of 35 units/ml, which corresponds to 3,500 cells/ml [2]. Further improvement of the assay improved the detection limit to 1,000 cells/ml. Fully automated systems in DNA isolation, PCR mixture preparation and rapid thermal cycling profile offer a quick and sensitive tool for quantifying circulating DNA in plasma. However, these systems require considerable amounts of plasma: reliable DNA extraction for RQ-PCR requires at least 200 µl of plasma, whereas only 25 µl suffices for ELISA.

Conclusion

To determine circulating DNA in plasma, appropriate sample handling is mandatory. RQ-PCR and ELISA techniques have applications in measuring circulating DNA. Assessment of circulatory DNA is a useful tool for predicting morbidity and mortality in patients admitted to the ICU.

Competing interests

The author declares that they have no competing interests.

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