# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**6,900**
Open access books available

**185,000**
International authors and editors

**200M**
Downloads

**154**
Countries delivered to

Our authors are among the

**TOP 1%**
most cited scientists

**12.2%**
Contributors from top 500 universities

CLARIVATE ANALYTICS
**BOOK CITATION INDEX**
INDEXED

**WEB OF SCIENCE**™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

## Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

# Cell-Free Nucleic Acids as Biomarkers of Biocompatibility in Dialytic Process

Marie Korabečná[1] and Aleš Hořínek[2]
*First School of Medicine, Charles University in Prague*
*Czech Republic*

## 1. Introduction

In this review we describe what is recently known about the origin of free nucleic acids in human circulation, which processes can cause the elevations of their total concentrations and the alterations in the ratios among different types of circulating nucleic acids in human plasma and serum. We focus on the inflammatory response and apoptosis with regard to changes in the quantity and quality of circulating nucleic acids pool. We discuss the use of cell-free nucleic acids as biomarkers in patients with renal failure not only in hemodialysis but also in peritoneal dialysis with regard to future perspectives on the field of cell-free nucleic acids.

## 2. Origin and clearance of free nucleic acids in human circulation

The phenomenon of cell-free DNA circulating in human plasma was discovered in 1948 (Mandel &, Metais, 1948,). The authors detected DNA and RNA in blood plasma of healthy control and patients. With regard to the lack of biological understanding of such a finding their work remained unnoticed. During next thirty years only two studies focusing on cell-free DNA appeared. The patients with lupus erythematosus (Tan et al., 1966, in Swarup & Rajeswari, 2007) and the patients with rheumatoid arthritis (Ayala et al., 1951) were studied and elevated levels DNA in circulation were reported. The medical importance of cell-free nucleic acids was recognized when the decreasing levels of these nucleic acids were reported in cancer patients after successful chemotherapy (Leon et al., 1977).

In 1994, mutated oncogene K-ras was discovered in the pool of circulating DNA in pancreatic cancer patients (Sorenson at al., 1994). In 1997 the circulating DNA of fetal origin was found in plasmas of pregnant women (Lo et al., 1997). Till today numerous studies were performed not only to understand the nature and biological meaning of cell-free DNAs and RNAs but mainly to establish their diagnostic use under different clinical conditions such as cancer, autoimmune disorders, pregnancy related disorders and trauma (for review Tong & Lo, 2006).

Although recent molecular biology and genetics employ very broad spectrum of sophisticated methods, the complete understanding of biology of cell-free nucleic acids was so far not achieved. As there are speculations concerning the regulatory function of circulating nucleic acids in plasma, the clinical laboratory research and practice stream to develop approaches allowing the analytic use of these molecules. The main sources of nucleic acids in plasma are necrosis, apoptosis and active release by living, non-apoptotic cells. All mentioned processes will be discussed in details in following sections. Cell-free

nucleic acids in human plasma present very heterogeneous material with heterogeneous function – there are for example fragments of genomic DNA, mitochondrial DNA, but also mRNAs and microRNA. The forms in which they circulate are studied also with regard to the development of effective extraction methods for clinical laboratories.

## 2.1 Necrosis and apoptosis as sources of cell-free DNA

From the methodological point of view, it is difficult to exactly distinguish between the apoptotic derived fraction of cell-free DNA and the necrotic-derived one in plasma. Veiko et al. (2008) developed a method for in vivo evaluation of cell death in patients with acute and/or chronic heart disease. The main parameters evaluated in the study by Veiko et al. were: total concentrations of cell-free DNA (cfDNA) in the blood (or serum), concentration of serum ribosomal repeat (rDNA), content of rDNA in total cfDNA, but also factors involved in clearance of cfDNA such as nuclease activity and anti-DNA antibodies.

The authors clearly demonstrated significant increase in the concentration of rDNA in the cfDNA pool in patients with acute myocardial infarction. Such an accumulation of rDNA within cfDNA may be caused by the resistance of rDNA to the fragmentation by serum endonucleases, because the nuclease activities in the serum of both acute and chronic coronary disease patients were elevated in comparison to healthy individuals. The titers of anti-DNA antibodies were also higher in the patients group. The anti-DNA antibodies were predominantly bound to cfDNA. It seems that the release of rDNA fragments into the blood may reflect cellular death in the body (Veiko et al., 2008).

Another clinical situation connected with massive cellular death is represented by the multiple-organ dysfunction syndrome (MODS). In MODS, the initial insult damages target organs and leads to tissue necrosis. The necrosis induces a systemic inflammatory response and an alteration of hemodynamics, microcirculation and oxygen metabolism. As a consequence, distant organs may be damaged by necrosis or apoptosis.

The prognostic role of elevated levels of plasma cfDNA in critically ill patients was demonstrated by Wijeratne et al. (2004). Pachl et al. (2005) developed an assay allowing to distinguish between DNA released from apoptotic and necrotic cells. The assay is based on electrophoretic separation of isolated plasma cfDNA fragments on agarose gel. The DNA from apoptotic cells (aDNA) is represented by fragments of typical size resembling the ladder on an electrophoretic gel, but DNA derived from necrotic cells (genomic - gDNA) does not provide this typical pattern when subjected to electrophoretic separation. The authors applied their assay on the samples of plasma cfDNA obtained from intensive care unit patients. They found that the contribution of aDNA to the amount of total plasma DNA in the critically ill patients was 16 fold greater than the contribution of gDNA from necrotic cells. The levels of aDNA were highest on the day of admission and declined thereafter, but the levels of gDNA altered in the opposite manner.

The concentration of apoptotic DNA in samples collected from patients on the day of admission significantly differentiated survivors and non-survivors (Pachl et al., 2005). The study by Pachl confirmed the results of previous research performed on rats (Guan et al., 2002). The most surprising fact in this context is represented by the finding of the highest concentration of cfDNA of apoptotic origin at the time of patient admission to the intensive care unit. The possible explanation for this fact can be found in the induction of apoptosis by the activation of the intrinsic pathway caused by the affection of mitochondria as a consequence of cellular damage caused by primary insult (Crouser et al., 2002).

According to the results obtained in an animal study (Guan et al., 2002) and the first human study (Pachl et al., 2005) it seems that primary insult induces apoptosis in the relationship to its severity and necrotic DNA originates from secondary organ damage therefore it can be detected with later onset during the course of the illness (Pachl et al., 2005).

Muscle injury caused by athletic overtraining leads also to elevation of plasma cfDNA concentrations as reported by Fatouros et al. (2006). The study demonstrated increase of cfDNA levels in proportion to training load. Overtraining causes an acute breakdown with subsequent repair of skeletal muscle and it is characterized by changes in the functionality of immune system resulting in increased susceptibility to infections. The correlation of plasma cfDNA with creatinine kinase but not with C-reactive protein was reported in this study.

The results achieved by Fatouros and coworkers are in good agreement with the results of an earlier study (Atamaniuk et al., 2004) in which long-distance runners were examined and 9- to 17.5 fold increase in concentrations of cfDNA was found immediately after the run. With regard to these reports, the extent of physical activities of examined subjects before the sampling procedure must be taken in account when evaluating the results of cfDNA based studies. The problems concerning the clearance of plasma cfDNA will be discussed in one of the following sections.

## 2.2 Active release of nucleic acids by cells

Necrosis and apoptosis were originally sought to be the only sources of cell-free nucleic acids in the circulation. The pioneer study by Stroun (2001a) clearly demonstrated the active release of fragmented DNA from cells in culture where apoptosis has been inhibited. Anker with coworkers (Anker et al., 1976) provided evidence about the release of newly synthesized DNA from human leucocytes. They reported that the release process is unrelated to cell death and is regulated by a homeostatic mechanism. Precursor incorporation into the DNA was inhibited by DNase, RNase, Pronase, and actinomycin D in their experiments.

The original hypothesis that the tumor specific cfDNA originates from lysis of tumor cells on the interface between the tumor and circulation is not more viable with regard to the high concentration of cancer specific cfDNA in circulation. Sorenson (1997, in Stroun et al., 2001a) calculated that 1000 cancer cell per milliliter would be necessary to provide the amount of DNA found in the plasma of pancreatic cancer patients. Later, the extraction methods for cfDNA were improved, and it has been demonstrated that the concentrations of cfDNA in plasma of cancer patients are even ten times higher than originally sought.

The tumors are not able to supply the circulation with 10 000 cells per each milliliter therefore the active release of nucleic acids by tumor cells is recently accepted as the main source of cell-free nucleic acids in cancer patients.

Cell-free DNA can occur in different forms in circulation. It may be transported by vesicle based particles, nucleosomes and virtosomes. The extracellular DNA bound in all mentioned structures forms the so called nucleome (Peters & Pretorius, 2011). The sequences contained in the nucleome were compared with genomic DNA sequences (Stroun et al., 2001; van der Vaart & Pretorius, 2008; Beck et al., 2009; Puszyk et al., 2009). Beck et al. studied the nucleome of 50 healthy individuals and concluded that practically no gene sequence is highly overrepresented here in comparison with genomic DNA.

The overrepresentation of non-coding sequences – *Alu* repeats – in the nucleome was repeatedly reported in studies of this type (Stroun et al., 2001; van der Vaart & Pretorius, 2008; Beck et al., 2009). Next generation sequencing (high-througput sequencing) has been used to analyze and compare the nucleome of healthy controls and patients with breast

cancer. The higher representation of certain repetetive elements in the nucleomes of breast cancer patients was found and the finding was validated with regard to the diagnostic use for staging and outcome prediction (Beck et al., 2010).

During apoptosis DNA and RNA are packed in separate apoptotic bodies which are rapidly ingested by adjacent cells and professional phagocytes. The contribution of apoptotic DNA under physiological conditions to the pool of cfDNA is probably insignificant because DNA from apoptotic bodies is completely digested by DNAseII in lysosomes of phagocyting cells (Peters & Pretorius, 2011).

Cell-free DNAs are mostly in complex with histones in the form of nucleosomes. The association with histones can play the key role in the translocation of cfDNA across cell membrane. The binding of cfDNA with histone H3K27me2b was described, this histone could be crucial for externalization and stabilization of cfDNA in plasma (Beck et al., 2009).

It is known for longer time that regulated release of newly synthetized DNA/RNA – lipoprotein complexes requires energy. The newly synthetized particles contain DNA, RNA DNA-dependent DNA and RNA polymerases, lipoproteins and are referred as virtosomes. Synthesis of DNA for virtosomes probably takes place mainly in the G0 or G1 phase - it seems not to be limited to the mitosis (Peters & Pretorius, 2011).

Not only cell-free DNA can be detected in human plasma, but there is also broad spectrum of mRNA and microRNA. Surprisingly, these RNA molecules due their relative stability can be also isolated from patient plasma and analyzed in clinical laboratories. Their relative stability within the plasma samples is linked to the form in which they are released into circulation. These RNA molecules are enclosed in small vesicles referred as exosomes. Exosomes are 40 - 100 nm membrane bound vesicles of endocytic origin secreted by most cell types *in vivo*. More than 2,300 proteins and 270 microRNAs have been linked with exosomes derived from different biological fluid (Taylor et al., 2011). Exosomes were found *in vivo* in body fluid such as blood, urine, amniotic fluid, malignant ascites, bronchoalveolar lavage fluid, synovial fluid and breast milk *(*Simpson et al., 2008).

Recently it is not exactly known, if exosomes contains DNA or not. According to the research done by García-Olmo et al. (2000) it seems that exosomes can also carry DNA containing complexes.

The advent of genomic and proteomic technologies contributed to the understanding of the molecular composition of exosomes, but their biological functions remain still unclear. It is becoming apparent that they may be involved in the transfer of both mRNA and microRNA to the distant target cells to modulate their expression and behavior. The secretion of exosomes by tumor cells and their implication in the transport and propagation of infectious cargo such as prions and retroviruses provide the evidence that exosomes are important participants of different pathological processes (Simpson at al., 2008).

The group of García-Olmo demonstrated in an animal study that plasma from tumor-bearing rats was able to stably transfect cultured cells. The authors proposed a hypothesis that the metastasis might occur via transfection of susceptible cells located in distant target organ with dominant oncogenes circulating in plasma (García- Olmo et al., 1999) and called such a putative phenomenon "genometastasis."

One of the most common alterations of tumor related cfDNA is its hypermethylation. The methylated DNA fragment have been shown to be taken up by HeLa and human umbilical vein endothelial cells twice as efficiently as unmethylated fragments (Skvortsova, Vlassov & Laktionov, 2008).

Recent progress in immunology suggests that nucleic acids are active modulators of the immune system. Both RNA and DNA molecules can be detected by specific receptors - the so called Toll-like receptors, RIG-I-like receptors and NOD-like receptors (Koyama S. et al., 2010). All above mentioned facts contribute to the understanding of cell-free nucleic acids in human plasma as important tools in complex regulatory mechanisms involved in homeostasis and immune response under frequently changing endo- and exogenous conditions.

## 2.3 Clearance of circulating nucleic acids in human plasma

Cell-free nucleic acids circulate in plasma in different forms as described in previous sections. Their clearance and turnover represent very probably complex mechanism which is known only superficially. The equilibrium between nucleome and genome forms probably the base of genetic homeostasis (Peters & Pretorius, 2011). The individual cells from different organs can not only contribute to the pool of nucleic acids in plasma but also take part in their turnover using them for regulation of intracellular events in variable extent depending on the cell type and physiological state. The live span of the individual molecules and complexes determines the area of their effect in which the group of cells can benefit from horizontal gene transfer (Peters & Pretorius, 2011).

Our study (Horinek et al., 2008) brought an indirect evidence for the existence of homeostatic mechanisms between nucleic acids released into circulation and taken up by cells or digested by nucleases. We measured the concentrations of total cfDNA and fetal cfDNA in plasma of healthy pregnant women during pregnancy. Although the concentrations of total cfDNA were kept at the same level through the entire pregnancy, the fractions of cfDNA of fetal origin in the pool of total maternal cfDNA elevated from the first trimestr toward the labor. Lo et al. (1999) reported that fetal DNA is undetectable 2 hours postpartum, with a mean half-life of 16.3 min.

Under pathological conditions, the clearance of cfDNA can be affected as demonstrated in the study by Lau et al. (2002) where higher half-life of fetal DNA in preeclamptic (median 114 min) women was observed compared to healthy controls (median 28 min). Impaired clearance of cfDNA can be expected under other pathological conditions, for example in cancer patients. It has been actually documented that the activities of nucleases in cancer patient are lowered (Cherepanova et al., 2008).

It seems that cfDNA in human circulation is present predominantly in the form of nucleosomes (Holdenrieder et al., 2001). Liver, macrophages and immune system are mainly involved in the clearance of DNA bounded in nucleosomes as has been documented in animal model (Burlingame et al., 1996). Kupfer cells are able to degrade the naked DNA on their surfaces in a saturable process (Gauthier et al., 1996; Kobayashi et al., 2001). Botezatu et al. (2000) demonstrated that kidney is not the main route of elimination of cfDNA from the body, only 0,5 % – 2% of the cfDNA passes from bloodstream through kidney into urine. This group showed the presence of Y- chromosome sequences in the urine of pregnant women carrying male fetuses and detected KRAS mutations in the urine of patients with pancreatic and colorectal cancers.

The study by Chan et al (2010) confirmed that transrenal excretion of cfDNA is very low. Sequences of the Epstein- Barr virus they are present in plasma of patients suffering with nasopharyngeal carcinoma in high concentrations were used as model system to make the detection of cfDNA in urine more robust. Only minor fraction of DNA representing EBV sequences was found in the urine of examined patients, this fraction represented 0,0028% - 0,00018% of the clearance of creatinine.

The finding that the cfDNA is stable for at least 4 hours in urine (Su et al., 2005) is important for management of clinical samples and translation medicine based on examination of cfDNA quantity and quality. From the results of our pilot study (Korabecna et al., 2011) it would appear that plasma DNAse II (which is not inhibited in blood samples stabilized by EDTA) makes only minor contribution to the degradation of circulating DNA *in vitro.* If heparin is used for sample stabilization nucleases are not inhibited and cfDNA is degraded very quickly.

## 3. Concentrations of cell-free nucleic acids in human plasma in health and disease

### 3.1 Cancer patients

Numerous studies provided evidence for the value of cfDNA quantification in cancer diagnostics and the therapy monitoring. Healthy individuals have in most cases lower cfDNA concentrations than cancer patients.

Today, the study of cell-free DNAs and RNAs in cancer patients is the most fertile area of free-nucleic aids research  (8180 articles for key words "cell free nucleic acids and cancer" on PubMed till April 2011)

The qualitative alterations reported in cfDNA in cancer patients include mutations of oncogenes and tumor suppressor genes, microsatellite alterations, promoter hypermethylation of different cancer related genes and mitochondrial variation. In plasma of cancer patients, the existence of tumor specific RNAs detectable with recent methods was reported.

Detection of circulating RNA in tumor biology has the advantage over DNA-based approaches as transcripts can be both tumor and tissue-specific and therefore the origin and location of the tumor can be followed. For comprehensive review concerning different aspects of the use of circulating nucleic acids as biomarkers in cancer see Vlassov, Laktionov & Rykova (2010).

### 3.2 Pregnancy related disorders

Qualitative analysis of fetal cfDNA in maternal circulation has been used for fetal gender determination using detection of Y- chromosomal specific sequences, for the detection of single gene mutations in fetuses with achondroplasia, myotonic dystrophy, congenital adrenal hyperplasia, thalassemia, cystic fibrosis and Huntington disease. Recently, the fetal cfDNA is routinely examined to detect the RhD positive fetuses in RhD negative mothers.

Quantitative aberrations in the concentrations of total levels of cfDNA were found to be associated with different pregnancy related disorders such as preeclampsia, various placental pathologies, preterm labor, intrauterine growth retardation, polyhydramnion, and ectopic pregnancy. Some studies described the elevated values of total cfDNA in maternal circulation in pregnancies with aneuploid fetuses, but such results were not satisfactory confirmed by others (for review Hořínek et al., 2008).

### 3.3 Other diseases

Elevated plasma levels of cfDNA have been reported to correlate with the severity of injury in patients with polytrauma (Lo et al., 2000; Rainer & Lam, 2006) and with the severity of stroke (Rainer & Lam, 2006). Another study proposed to use the concentrations of cfDNA in plasma for outcome prognosis in acute myocardial infarction (Antonatos et al., 2006). Strong correlations were also found between increase of cfDNA in plasma and the outcome of intensive care unit patients (Rhodes et al., 2006; Saukkonen et al., 2007).

The elevated plasma levels of cfDNA were described to be an excellent marker for graft rejection in patients after renal transplantation (García-Moreira et al., 2010).

### 3.4 Inflammatory response

Due to well documented increase in cfDNA levels during inflammatory process (Fatouros et al., 2006) it is necessary to take in account the actual health status of control subjects and patients with complex clinical diagnosis. The elevated levels of cfDNA interpreted without the clinical context can not indicate pathological situations. The actual physical activity of examined subjects may also contribute to the elevations of total cfDNA in plasma and serum (Atamaniuk et al., 2004; Fatouros et al., 2006).

The mechanism of immune response to increased amount of nucleosomes in circulation was examined in systemic lupus erythematosus (SLE). In this rheumatic autoimmune disease of unknown etiology numerous autoantibodies against circulating nucleosomes are produced. The production of anti-dsDNA autobodies serves as disease marker for SLE.

Polymorphonuclear cells, dendritic cells and monocytes strongly bind nucleosomes on their surfaces, the binding of nuckeosomes to lymphocytes is weaker. Circulating nucleosomes are endocyted in polymorphonuclears, dendritic cells and monocytes. It has been shown in polymorphonuclear cells that the nucleosomes are not translocated into nucleus. Polymorphonuclear cells represent the link between innate and adaptive immunity therefore the perturbations in the regulation of their function could lead to the development of autoimmunity (Lindau et al., 2011).

The role of the receptor TLR9 in the binding of circulating DNA and development of SLE is not fully understood. It is known that the TLR9 plays an important role in recognition of dsDNA in extracellular space and in its internalization (Lindau et al., 2011) but the internalized DNA molecules may stimulate the production of IFN I in dendritic cells also in the TLR9–independent pathway (Martin & Elkon, 2006).

After a hemodialytic procedure, the concentrations of cfDNA in patients are dramatically elevated as has been demonstrated in numerous studies (Atamaniuk et al., 2006; Garcia-Moreira et al., 2006; Korabecna et al., 2008; Horinek et al. 2011). Garcia-Moreira et al. reported quite rapid normalization to normal values during 30 min after the end of the procedure. The problems associated with the well documented increase of cfDNA during the dialytic procedure will be discussed in the following sections.

The interpretations of each cfDNA based study must be performed carefully with the respect to clinical data concerning the presence of autoimmune disease, malignancy, renal transplantation or acute infection in examined subjects. All mentioned conditions must be used as exclusion criteria.

For total plasma concentrations of different types of circulating ribonucleic acids, similar clinical data are urgently needed.

## 4. Cell-free nucleic acids as biomarkers in dialytic process

During hemodialysis, the interaction of dialyzer membrane with patient´s blood leads to the activation of alternate pathway of complement. The biologically active complement component C5A is generated and activates the aggregation of neutrophils and their adherence to the endothelial surfaces. Neutrophils harvested during hemodialysis exhibit altered oxidative response, chemotaxis, aggregation, and adherence (Lewis & Van Epps,

1987). Neutrophils have short half-live - under normal conditions they spend approximately 12h in the circulation and then migrate into normal tissues or are attracted by chemotactic stimuli to inflamed tissues. Once in tissues, they do not return back to circulation but undergo apoptosis and are cleared by phagocytosis (Savill 1997).

Direct contact of cell with membranes in dialyzer can also induce apoptosis (Carracedo et al., 1995). Interaction of cell-surface protein of monocytes with dialysis membrane can stimulate mononuclear cells to interact with neutrophils and induce their apoptosis (Nahar et al., 2001). Interaction between dialysis membrane and blood leads not only to complement activation but also to cytokine synthesis and release. Certain cytokines, such as tumor necrosis factor (TNF) and interleukin (IL)-1β 1, component C5a and bacterial lipopolysacchride (LPS) can increase neutrophil survival (Colotta et al., 1992; Lee et al., 1993).

As some interleukins are generated during hemodialysis and activation of monocytes occurs it seems that hemodialysis effects are comparable to the pathophysiological mechanisms of inflammation and hypersensitivity response (García-Moreira et al., 2006).

The survival of leucocytes during hemodialysis is influenced by numerous factors therefore an analyte which would mirror the complex character of biological events during hemodialysis with regard to the impact of the procedure on immune system of each individual patient may be very helpful. The study of cell-free nucleic acids seems to be a promising approach. In the next sections, the first attempts in this newly arising field will be presented.

## 4.1 Cell-free nucleic acids in hemodialyzed patients

The first study focused on the examination of cfDNA was carried out in 1977 by means of semiquantitative counter-immunoelectrophoresis (Steinman & Ackad, 1977). It is till today the only one study examining the appearance of cfDNA in vitro experiment using circulation of fresh blood through dialysis coil. The authors conclude that the passage of blood through the coil itself can account, at least in part, for circulating DNA and speculate about the leucocytes as the main source of this DNA.

Fournie et al. (1989) used the detection method based on incorporation of radiolabeled nucleotide in nick translation reaction and confirmed the results obtained by Steinman & Ackad (1977). In the study by Fournie and coauthors, 45 patients during 99 sessions of hemodialysis or hemofiltration were followed. Independently on the method of treatment, during the first 3 h of the session the elevations of plasma DNA were observed. The authors concluded that the dialytic procedure induces the death of leucocytes on the artificial surfaces and the release of their DNA into blood thus is responsible for elevated values of extracellular DNA. In this study plasma DNA levels were increased in interdialytic interval in 41 out of 99 samples, among samples with elevated cfDNA levels were 18 out of 24 samples collected from hepatitis B infected person.

The study performed by Rumore et al. (1992) used the same cfDNA detection method as Steinman & Ackad (1977) and exploited the possibilities of the visualization of radiolabeled DNA on electrophoretic gels to demonstrate that in the blood of hemodialyzed patients the electrophoretic patterns known from patients with lupus erythematosus (SLE) are clearly recognizable. This study documented also by immunoprecipitation that at least the large fraction of cfDNA in the blood of hemodialyzed patients circulates in the form of nucleosomes like in the patients with lupus erythematosus. The autoradiograms provided the picture of DNA samples fragmented in the typical manner with predominance of bands corresponding to DNA of 150-200, 400 and in some cases 600 bp.

The authors calculated also very carefully the half-live of this fraction of cfDNA and its rate of clearance. Their study was limited by selection of patients with postdialytic concentrations of cfDNA high inough for performance of experiments. The half-life of cfDNA in blood of hemodialyzed patients immediately after hemodialysis was determined as 4 minutes in this study. The rate of cfDNA clearance from circulation calculated by authors is equivalent to the DNA content of approximately 15 g of a solid tissue such as liver or of the leucocyte content of 600 ml of whole blood at WBC of 5000/mm³ per day (Rumore et al., 1992).

Although the nucleosomes were found in the blood of patients after hemodialysis in similar amount as in the patients with lupus erythematosus, the hemodialysis itself does not induce any autoimmune response. According to their results, Rumore et al. (1992) hypothesized that in patients with lupus erythematosus an impairment of mechanism of nucleosome clearance contribute to the development of autoimmune disease.

Atamaniuk et al. (2006) investigated the levels of cfDNA in hemodialyzed patients together with markers of early and late apoptosis in leucocytes. For detection of early and late apoptosis flow cytometric measurements of annexin V expression in combination with 7-amino-actinomycin D (7AAD) were used. Annnexin V has high affinity for phosphatidylserine which is translocated during early apoptosis from the inner to the outer surface of the plasma membrane. The nuclear 7AAD staining is possible only in the late apoptosis or in dead cells because the plasma membrane is desintegrated in such stages and allows the throughput of the stain into the nucleus. Employing this methodology, the leucocyte can be classified as normal (annexin V- and 7AAD-), early apoptotic (annexin V+ and 7AAD -), and apoptotic (annexinV + and 7AAD+). Ten patients hemodialyzed on synthetic polymer membranes [Fresenius Polysulfone Capillary dialyzers (F6) low-flux] and 30 healthy subjects were examined in the study. Blood samples from hemodialyzed patients were obtained before hemodialysis (HD), after 20 min of HD, at the end of HD. The concentrations of cfDNA in plasma samples were measured using Vistra Green and human placental DNA calibrators. The emitted fluorescent signals were measured in the LightCycler at 530 nm and reported in picograms per microliters.

In HD patients the cfDNA levels before hemodialysis were slightly higher than in controls. The significant increase was detected after 20 min of HD. The highest values of cfDNA concentrations were determined at the end of HD. The cfDNA samples isolated from plasma of patients were subjected to agarose gel electrophoresis. In pre-HD samples and in samples taken after 20 min of HD the weaker ladders than in samples after HD were observed.

The fraction of leucocytes in early apoptosis was significantly higher in predialytic interval in patients than in controls. The number of apoptotic leucocytes increased during HD. The authors conclude that the apoptosis induced by the contact of leucocytes with dialysis membrane may be the main source of elevated concentrations of cfDNA during HD sessions.

Different types of dialysis membrane were examined in the study by García-Moreira et al. (2006) performed with regard to their influence on the cfDNA levels in plasma. In this study, concentrations of cfDNA were measured first time using real-time quantitative PCR. The sequence of β-*globin* gene was amplified to quantify the total amount of cfDNA in plasma samples. To establish the range of normal values, the samples from 100 healthy voluntary blood donors were examined.

Thirty patients on regular HD were studied in 52 sessions. Chronic renal failure in patients was caused by diabetic nephropathy (7 patients), glomerulosclerosis (6 patients), nephroangioesclerosis (6 patients), chronic pyelonephritis (3 patients), polycystic kidney

disease (2 patients), primary amyloidosis (1 patient), renal artery stenosis (1 patient), renal transplantation (1patient), and unknown causes (3 patients).The study was designed to compare high-flux (n=37) vs. low-flux (n=15) and polysulfone (n=42) vs. modified cellulose (n=10) membranes, seven different membranes were used: Arylane H, Tricea 210, HF-80, Arylane M6, FX-60, FX-80, and P160. The effect of the dialysis length on cfDNA concentration after HD was evaluated.

Although correct interpretation of the results is difficult with regard to many variable factors, cfDNA levels increased more than four-fold in 75% of the patients after HD. The study by García-Moreira et al. (2006) has two priorities: 1/ the real-time PCR method was used for the first time to quantify cfDNA; 2/ clear data about the clearance of elevated cfDNA concentrations after HD were provided to scientific community. The authors reported a rapid decline to normal values within 30 minutes of completing a hemodialysis session.

In our study (Korabecna et al., 2008), we determined not only pre- and post-dialytic cfDNA concentrations in patients on hemodialysis (n=17) but we also examined non-dialyzed patients with chronic kidney disease (n=20) and patients on peritoneal dialysis (n=18) in comparison with healthy volunteers (n=20). We measured the total cfDNA levels using real-time PCR on GADPH gene. All patients involved in our study were hemodialyzed on high-flux polysulfone membrane.

In HD patients, we found elevated postdialytic cfDNA values and reported high interindividual variability. We found also patients with lowered cfDNA values after hemodialysis. The levels of cfDNA in HD patients in pre-dialytic interval were significantly increased when compared with patients with chronic kidney disease and patients on peritoneal dialysis but there was no significant difference in comparison with healthy persons probably due to the very broad range of cfDNA concentrations obtained in our control group.

It seems that the study of quantitative and qualitative cfDNA parameters with regard to the process of hemodialysis can bring very interesting insights into the biological aspect of the treatment with important clinical consequences.

### 4.2 Cell-free nucleic acids in patients on peritoneal dialysis

In our study mentioned above (Korabecna et al., 208), we use real-time PCR based on the GAPDH gene sequence amplification for quantification of cfDNA in plasma and overnight dialysate in patients on peritoneal dialysis. We determined the ratio of dialysate cfDNA/plasma cfDNA (P/D ratio) and discovered that this ratio inversely correlates with the duration of period for which the person is treated by peritoneal dialysis. The values of P/D ratio lower than one were found in patients dialysed for longer time (median 17 months).

Ozkaya et al. (2009) studied the plasma cfDNA in children on peritoneal dialysis and found significantly elevated values in comparison to healthy children and positive correlation with C- reactive protein levels in treated children.

Samples of peritoneal effluent with regard to their content of cfDNA were examined in the study by Pajek et al. (2009) to analyze the impact of PD solution with different biocompatibility and cytotoxic properties on the peritoneal membrane cells. Two PD solutions were tested: a conventional lactate buffered, acidic solution and a novel, bicarbonate/lactate buffered, neutral solution low in glucose degradation products. A significant decrease in appearance of cfDNA in effluent was observed with the novel PD solution.

## 5. Future perspectives

The study of cell-free nucleic acids in connection with hemodialysis promises new clinically important findings. Application of new methods – mainly the technology of Next Generation Sequencing (NGS) in combination with bionformatics – will bring huge amount of qualitative and quantitative data and allow the study of tiny fractions of specific sequences on the total circulating nucleic acids background. Recently, no studies focused on analysis of microRNA and mRNA in the plasma of hemodialyzed patient are available. Probably, such studies will be managed in near future.

It has been reported that some interleukins are generated during hemodialysis. It is known that activated monocytes during HD liberate certain mediators of immune response (Kim et al., 2011) therefore the study of individual mechanisms of immune response in patients may be very helpful in the process of individualized treatment. Insights into the current character of immune response can be achieved through the analysis of the methylation status of selected genes using existing arrays technologies. Such high throughput technologies will lead to the comparison of hemodialysis effects on the character of immune response in different groups of patients with different comorbidities.

DNA methylation patterns determined at the level of cfDNA in hemodialyzed patiens may be predictive of response to specific drugs and drug combinations.

The clinical studies performed on hemodialyzed patients can also improve the theoretical knowledge about the clearance and turnover of circulating nucleic acids in connection to the regulatory functions of this extremely interesting class of molecules.

## 6. Conclusion

Today, there are various methodological approaches allowing the quantitative and qualitative characterization of cell-free nucleic acids in circulation of hemodialyzed patients. The study od cfDNA in plasma during the hemodialysis began 30 years ago and brought interesting results concerning the apoptotic origin of at least an important fraction of this DNA and increase of its plasma concentration during the hemodialysis session regardless of the used membranes and duration of session. In the past decade, the clearance of cfDNA from plasma was studied not only in hemodialyzed patients, and it was concluded, that it is very rapid (the half-life is given in minutes) and kidney does not play the major role in this process. In last years, the attention is paid to the examination of cell-free ribonucleic acids in the circulation with regard to their use as biomarkers under different pathological conditions. The application of such methodologies in the field of hemodialysis is still awaiting for the researchers, as well as the application of high throughput technologies likes Next Generation Sequencing and array technologies of different design. The connection of new technologies with clinical studies will result not only in clinical benefit but also in deeper insight into basic biological mechanisms of intracellular communication, epigenetic regulation  and modulation of immune function at the individualized level leading to truly personalized medicine.
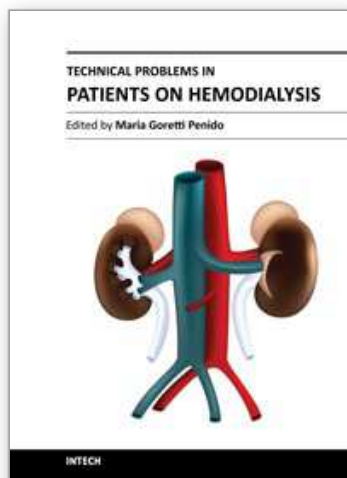
## 7. Acknowledgment

## 8. References

Anker, P.; Stroun, M.; Maurice, P.A. (1976). Spontaneous Extracellular Synthesis of DNA Released by Human Blood Lymphocytes. *Cancer Research*, Vol. 36, No. 4, (August 1976), pp. 2832-2839, ISSN 0008-5472

Antonatos, D.; Patsilinakos, S.; Spanodimos, S.; Korkonikitas, P.; Tsigas, D. (2006). Cell-Free DNA Levels as a Prognostic Marker in Acute Myocardial Infarction. *Annals of New York Academy of Science*, Vol. 1075 (September 2006), pp. 278-281, ISSN 00778923

Atamaniuk, J.; Vidotto, C.; Tschan, H.; Bachl, N.; Stuhlmeier, K.M.; Müller, M.M. (2004) Increased Concentrations of Cell-Free Plasma DNA after Exhaustive Exercise. *Clinical Chemistry*, Vol. 50, No. 9, (September 2004), pp. 523–526, ISSN 0009-9147

Atamaniuk, J.; Ruzicka, K.; Stuhlmeier, K.M.; Karimi, A.; Eigner, M.; Mueller, M.M. (2006). Cell-Free Plasma DNA: a Marker for Apoptosis During Hemodialysis. *Clinical Chemistry*, Vol. 52, No. 3, (March 2006), pp. 523–526, ISSN 0009-9147

Ayala, W.; Moore, L.W. & Hess, E.L. (1951). The Purple Color Reaction Given by Diphenylamine Reagent. I. With Normal and Rheumatic Fever Sera. *Journal of Clinical Investigation*, Vol. 30, No. 7, (July 1951), pp. 781-785, ISSN 0021-9738

Beck, J.; Urnovitz, H.B.; Riggert, J.; Clerici, M.; Schutz, E. (2009). Profile of the Circulating DNA in Apparently Healthy Individuals. *Clinical Chemistry*, Vol. 55, No. 4, (April 2009), pp. 730–738, ISSN 0009-9147

Beck, J.; Urnovitz, H.B.; Mitchell, W.M.; Schütz, E. (2010). Next Generation Sequencing of Serum Circulating Nucleic Acids from Patients with Invasive Ductal Breast Cancer Reveals Differences to Healthy and Nonmalignant Controls. *Molecular Cancer Research*, Vol. 8, No. 3, (March 2010), pp. 335-342, ISSN 1541-7786

Botezatu, I.; Serdyuk, O.; Potapova, G.; Shelepov, V.; Alechina, R.; Molyaka, Y.; Ananév, V.; Bazin, I.; Garin, A.; Narimanov, M.; Knysh, V.; Melkonyan, H.; Umansky, S.; Lichtenstein, A.(2000). Genetic Analysis of DNA Excreted in Urine: a New Approach for Detecting Specific Genomic DNA Sequences from Cells Dying in an Organism. *Clinical Chemistry*, Vol. 46, No. 8, (August 2000), pp. 1078–1084, ISSN 0009-9147

Burlingame, R.W.; Volzer, M.A.; Harris, J.; Du Clos, T.W. (1996). The Effect of Acute Phase Proteins on Clearance of Chromatin from the Circulation of Normal Mice. *Journal of Immunology*, Vol. 156, No. 12, (June 1996), pp. 4783–4788, ISSN 0022-1767

Carracedo, J.; Ramirez, R.; Pintado, O.; Gomez-Villamandos, J.C.; Martin-Malo, A.; Rodriguez, M.; Aljama.P. (1995). Cell Aggregation and Apoptosis Induced by Hemodialysis Membranes. *Journal of the American Society of Nephrology*, Vol. 9, No.1, (January 1995), pp. 1586–1591, ISSN 1046-6673

Chan, K.C.; Leung, S.F.; Yeung, S.W.; Chan, A.T.; Lo, Y.M. (2008). Quantitative Analysis of the Transrenal Excretion of Circulating EBV DNA in Nasopharyngeal Carcinoma Patients. *Clinical Cancer Research*, Vol. 1, No. 14(15), (August 2008), pp. 4809-4813, ISSN 1078-0432

Cherepanova. A.V.; Tamkovich, S.N.; Bryzgunova, O.E.; Vlassov, V.V.; Laktionov, P.P. (2008) Deoxyribonuclease Activity and Circulating DNA Concentration in Blood Plasma of Patients with Prostate Tumors. *Annals of Nex York Academy of Sciences*, Vol. 1137, (August 2008) pp. 218-221, ISSN 00778923

Colotta, F.; Re, F.; Polentarutti, N.; Sozzani, S.; Mantovami, A. (1992). Modulation of Granulocyte Survival and Programmed Cell Death by Cytokines and Bacterial Products. *Blood*, Vol. 80, No. 8, (October 1992), pp. 2012–2020, ISSN 0006-4971

Crouser, E.D.; Julian, M.W. & Blaho, D.V. (2002). Endotoxin-Induced Mitochondrial Damage Correlates with Impaired Respiratory Activity. *Critical Care Medicine*, Vol. 30, No. 2, (February 2002), pp. 276-284, ISSN 0090-3493

Fatouros, I.G.; Destouni, A.; Margonis, K.; Jamurtas, A.Z.; Vrettou, C.; Kouretas, D., Mastorakos, G.; Mitrakou, A.; Taxildaris, K.; Kanavakis, E.; Papassotiriou, I. (2006). Cell-Free Plasma DNA as a Novel Marker of Aseptic Inflammation Severity Related to Exercise Overtraining. *Clinical Chemistry*, Vol. 52, No. 9, (September 2006), pp. 1820–1824, ISSN 0009-9147

Fournie, G.J.; Lule, J.; Dueymes, J.M.; Laval, F.; Delobbe, I.; Verner. I., Pourrat, J.P. (1989). Plasma DNA in Patients Undergoing Hemodialysis or Hemofiltration: Cytolysis in Artificial Kidney is Responsible for the Release of DNA in Circulation. *American Journal of Nephrology* , Vol. 9, No. 5, pp. 384–391, ISSN 0250-8095

García-Olmo, D.; García-Olmo, D.C.; Ontañón, J.; Martinez, E.; Vallejo, M. (1999). Tumor DNA Circulating in the Plasma Might Play a Role in Metastasis. The Hypothesis of the Genometastasis. *Histology and  Histopathology*, Vol. 14, No. 4, (October 1999), pp. 1159-1164, ISSN 0213-3911

García-Olmo, D.; García-Olmo, D.C.; Ontañón, J.; Martinez, E. (2000). Horizontal Transfer of DNA and the "Genometastasis Hypothesis". *Blood*, Vo. 95, No. 2, (January 2000), pp. 724-725, ISSN 0006-4971

García-Moreira, V.; Prieto García, B.; Baltar Martín, J.M.; Ortega Suárez, F.; Alvarez, F.V. (2009). Cell-Free DNA as a Noninvasive Acute Rejection Marker in Renal Transplantation.  *Clinical Chemistry*, Vol. 55, No. 11, (November 2009), pp. 1958-1966, ISSN 0009-9147

Gauthier, V.J.; Tyler, L.N.; Mannik, M. (1996). Blood Clearance Kinetics and Liver Uptake of Mononucleosomes in Mice. *Journal of Immunology*, Vol. 156, No. 3, ( February 1996), pp. 1151–1156, ISSN 0022-1767

Guan, J.; Jin D.D.; Jin, L.J. (2002). Apoptosis in Organs of Rats in Early Stage after Polytrauma Combined with Shock. *Journal of Trauma,* Vol. 52, No 1, (January 2002), pp, 104-111, ISSN 1529-8809

Holdenrieder, S.; Stieber, P.; Bodenmuller, H.; Busch, M.; Von Pawel, J.; Schalhorn, A.; Nagel. D.; Seidel, D. (2001). Circulating Nucleosomes in Serum. *Annals of New York Academy of Sciences,* Vol.  945, (September 2001), pp. 93–102, ISSN 00778923

Horinek, A.; Korabecna, M.; Panczak, A.; Ulcova Gallova. Z.; Nouzova, K.; Calda, P.; Hancarova. M. (2008). Cell-Free Fetal DNA in Maternal Plasma During Physiological Single Male Pregnancies: Methodology Issues and Kinetics. *Fetal Diagnosis and Therapy*, Vol. 24, No. 1, (May 2008), pp. 15-21, ISSN 1015-3837

Horinek , A.; Panczak, A.; Mokrejsova, A.; Rocinova, K.; Korabecna, M; Cerny, D.; Tesar, V. (2011). Comparison of Plasma Cell-Free DNA Levels with Gene Expression Profiles of Peripheral Blood Cells During Hemodialysis. In: *Circulating Nucleic Acids in Plasma and Serum*, P.Gahan, (Ed.), 159-163, Springer, ISBN 978-90-481-9381-3

Kobayashi, N.; Kuramoto, T.; Yamaoka, K.; Hashida, M.; Takakura,Y. (2001). Hepatic Uptake and Gene Expression Mechanisms Following Intravenous Administration of Plastid DNA by Conventional and Hydrodynamics-Based Procedures. *Journal of*

*Pharmacology and Experimental Therapeutics*, Vol. 297, No. 3, (June 2001), pp. 853–860, ISSN 0022-3565

Kim, H.W.; Yang, H.N.; Kim, M.G.; Choi, H.M.; Jo, S.K.; Cho, W.Y.; Kim, H.K. (2011). Microinflammation in Hemodialysis Patients Is Associated with Increased CD14CD16(+) Pro-Inflammatory Monocytes: Possible Modification by On-Line Hemodiafiltration. *Blood Purification*, Vol 31, No. 4. (January 2011) pp. 281-288, ISSN 0253-5068

Korabecna, M.; Opatrna, S.; Wirth, J.; Rulcova, K.; Eiselt, J.; Sefrna, F.; Horinek, A. (2008). Cell-Free Plasma DNA During Peritoneal Dialysis and Hemodialysis and in Patients with Chronic Kidney Disease. *Annals of New York Academy of Sciences*, Vol. 1137, (August 2008), pp. 296-301, ISSN 00778923

Korabecna, M.; Horinek, A.; Bila, N.; Opatrna, S. (2011). Circadian Rhytmicity and Clearance of Cell-Free DNA in Human Plasma. In: *Circulating Nucleic Acids in Plasma and Serum*, P.Gahan, (Ed.), 159-163, Springer, ISBN 978-90-481-9381-3

Koyama, S.; Akira, S.; Ishii, K.J. (2010) Immune Recognition of Nucleic Acids and Their Metabolites. In: *Extracellular Nucleic Acids*, E.Y, Rykova, Y. Kikuchi (Eds.), Springer ISBN: 3642126162, Berlin, Germany

Lau, T.W.; Leung, T.N.; Chan, L.Y.; Lau, T.K.; Chan, K.C.; Tam, W.H.; Lo, Y.M. (2002). DNA Clearance from Maternal Plasma is Impaired in Preeclampsia. *Clinical Chemistry*, Vol. 48, No. 12, (December 2002), pp. 2141–2146, ISSN 0009-9147

Lee, A.; Chyte M.K.B. & Haslett, C. (1993). Inhibition of Apoptosis and Prolongation of Neutrophil Functional Longevity by Inflammatory Mediators. *Journal of Leukocyte Biology*, Vol. 54, No. 4, (October 1993), pp. 283–288, ISSN 0741-5400

Leon, S.A.; Shapiro, B.; Sklaroff, D.M.; Yaros, M.J. (1977). Free DNA in the Serum of Cancer Patients and the Effect of Therapy. *Cancer Research*, Vol. 37, No. 3, (March 1977), pp. 646-650, ISSN 0008-5472

Lewis, S.L. & Van Epps, D.E. (1987). Neutrophil and Monocyte Alterations in Chronic Dialysis Patients. *American Journal of Kidney Diseases*, Vol. 9, No. 5, (May 1987), pp. 381–395, ISSN 0272-6386

Lindau, D.; Rönnefarth, V.; Erbacher, A.; Rammensee, H.G.; Decker, P. (2011). Nucleosome-Induced Neutrophil Activation Occurs Independently of TLR9 and Endosomal Acidification: Implications for Systemic Lupus Erythematosus. *European Journal of Immunology*, Vol. 41, No. 3, (March 2011), pp. 669-681, ISSN 0014-2980

Lo, Y.M.; Corbetta, N.; Chamberlain, P.F.; Rai, V.; Sargent, I.L.; Redman, C.W.; Wainscoat, J.S. (1997). Presence of Fetal DNA in Maternal Plasma and Serum. *Lancet*, Vol 350. No. 9076, (August 1997), pp. 350-487, ISSN 0140-6736

Lo Y.M.D.; Zhang, J.; Leung, T.N.; Lau, T.K.; Chang, A.M.; Hjelm, N.M. (1999) Rapid Clearance of Fetal DNA from Maternal Plasma. *American Journal of Human Genetics*, Vol. 64, No. 1, (January 1999), pp. 218–224, ISSN 0002-9297

Lo, Y.M.; Rainer, T.H.; Chan, L.Y.; Hjelm, N.M.; Cocks, R.A. (2000). Plasma DNA as a Prognostic Marker in Trauma Patients. *Clinical Chemistry*, Vol. 46, No. 3, (March 2000), pp. 319–323, ISSN 0009-9147

Martin, D. A. & Elkon, K. B. (2006). Intracellular Mammalian DNA Stimulates Myeloid Dendritic Cells to Produce Type I Interferons Predominantly Through a Toll-Like Receptor 9-Independent Pathway. *Arthritis and Rheumatism*, Vol 54, No. 3, (March 2006), pp. 951–962, ISSN 0004-3591

García-Moreira, V.; de la Cera Martínez, T.; Gago González, E.; Prieto García, B.; Alvarez Menéndez, F.V. (2006). Increase in and Clearance of Cell-Free Plasma DNA in Hemodialysis Quantified by Real-Time PCR. *Clinical Chemistry and Laboratory Medicine*, Vol. 44, No. 12, pp.1410–1415, ISSN 1434-6621

Nahar, N.; Shah, H.; Siu, J.; Colvin, R.; Bhaskaran, M.; Ranjan, R.; Wagner, J.D.; Singhal, P.C. (2001). Dialysis Membrane-Induced Neutrophil Apoptosis is Mediated Through Free Radicals. *Clinical Nephrology*, Vol. 56, No. 1, (July 2001), pp. 52–59, ISSN 0301-0430

Pachl, J.; Duska, F.; Waldauf, P.; Fric, M.; Fanta, J.; Zdárský, E. (2005). Apoptosis as an Early Event in the Development of Multiple Organ Failure? *Physiological Research*, Vol. 54, No. 2, (2005), pp. 697-699, ISSN 0862-8408

Pajek, J.; Kveder, R.; Gucek, A.; Skoberne, A.; Bren, A.; Bucar, M.; Cerne, D.; Lukac-Bajalo, J. (2010). Cell-free DNA in the Peritoneal Effluent of Peritoneal Dialysis Solutions. *Therapeutic Apheresis and Dialysis*, Vol. 14, No. 1, (February 2010), pp. 20-26, ISSN 1744-9979

Peters, D.L. & Pretorius, P.J. (2011). Origin, Translocation and Destination of Extracellular Occurring DNA - A new Paradigm in Genetic Behaviour. *Clinica Chimica Acta*, Vol. 412, No. 11-12, (May 2011), pp. 806-811, ISSN 0009-8981

Puszyk, W.M.; Crea, F.; Old, R.W. (2009). Unequal Representation of Different Unique Genomic DNA Sequences in the Cell-Free Plasma DNA of Individual Donors. *Clinical Biochemistry*, Vol. 42, No. 7-8, (May 2009), pp. 736–738, ISSN 0009-9120

Rainer, T.H. & Lam, N.Y.L. (2006). Circulating Nucleic Acids and Critical Illness. *Annals of New York Academy of Sciences*. Vol. 1075, (September 2006), pp. 271–277 , ISSN 00778923

Rhodes, A.; Wort, S.J.; Thomas, H.; Collinson, P.; Bennett, E.D. (2006). Plasma DNA Concentration as a Predictor of Mortality and Sepsis in Critically Ill Patients. *Critical Care*. Vol 10, No. 2, (April 2006), pp. R60, ISSN 1466-609X

Rumore, P.; Muralidhar, B.; Lin, M.; Lai, C.; Steinman, C.R. (1992). Haemodialysis as a Model for Studying Endogenous Plasma DNA: Oligonucleosome-Like Structure and Clearance. *Clinical and Experimental Immunology*, Vol. 90., No. 1, (October 1992), pp. 56-62, ISSN 0009-9104

Saukkonnen, K.; Lakkisto, P.; Varpula, M.; Varpula, T.; Voipio-Pulkki, L.M.; Pettilä, V.; Pulkki, K. (2007). Association of Cell-Free Plasma DNA with Hospital Mortality and Organ Dysfunction in Intensive Care Unit Patients. *Intensive Care Medicine*, Vol. 33, No. 9, (September 2007), pp. 1624–1627, ISSN 0342-4642

Savill, J. (1997). Apoptosis in Resolution of Inflammation. *Journal of Leukocyte Biology*, Vol 61, No. 4, (April 1997), pp. 375–380, ISSN 0741-5400

Simpson, R.J.; Jensen, S.S; Lim. JW. (2008). Proteomic Profiling of Exosomes: Current Perspectives. *Proteomics*, Vol. 8, No.19, (October 2008 ), pp. 4083-4099, ISSN 1615-9853

Skvortsova, T.E.; Vlassov, V.V., Laktionov, P.P. (2008). Binding and Penetration of Methylated DNA into Primary and Transformed Human Cells. *Annals of New York Academy of Sciences*, Vol. 1137, (August 2008), pp. 36-40, ISSN 00778923

Sorenson, G.D.; Pribish, D.M.; Valone, F.H.; Nemolí, V.A.; Bzik, D.J.; Yao, S.L. (1994). Soluble Normal and Mutated DNA Sequences From Single-Copy Genes in Human Blood.

*Cancer Epidemiology, Biomarkers & Prevention*, Vol. 3, No. 1, (January-February 1994), pp. 67-71, ISSN 1055-9965

Steinman, C.R. &, Ackad A. (1977). Appearance of Circulating DNA During Hemodialysis. *American Journal of Medicine*, Vol. 62, No. 5, (May 1977), pp. 693–697, ISSN 0002-9343

Stroun, M.; Lyautey, J.; Lederrey, C.; Olson-Sand, A.; Anker, P. (2001a). About the Possible Origin and Mechanism of Circulating DNA Apoptosis and Active DNA Release. *Clinica Chimica Acta*. Vol. 313, No. 1-2, (November 2001), pp. 139-142, ISSN 0009-8981

Stroun, M.; Lyautey, J.; Lederrey, C.; Mulcahy, H.E.; Anker, P. (2001b). Alu Repeat Sequences are Present in Increased Proportions Compared to a Unique Gene in Plasma/Serum DNA: Evidence for a Preferential Release from Viable Cells? *Annals of New York Academy of Sciences*, Vol. 945, ( September 2001),  pp. 258-264, ISSN 00778923

Su,Y.H.; Wang, M.; Aiamkitsumrit, B.; Brenner, D.E.; Block, T.M. (2005). Detection of a K-ras Mutation in Urine of Patients with Colorectal Cancer. *Cancer Biomarkers*, Vol. 1, No. 2-3, (August 2005), pp.177-182, ISSN 1574-0153

Swarup, V. & Rajeswari, M.R. (2007). Circulating (Cell-Free) Nucleic Acids - a Promising, Non-Invasive Tool for Early Detection of Several Human Diseases. *FEBS Letters*, Vol. 581, No. 5, (March 2007), pp. 795-799, ISSN 0014-5793

Taylor, D.D.; Zacharias, W.; Gercel-Taylor, C. (2011) Exosome Isolation for Proteomic Analyses and RNA Profiling. *Methods in Molecular Biology*, Vol. 728, (2011), pp. 235-246. ISSN 1064-3745

Tong, Y.K. & Lo, Y.M. (2006). Diagnostic Developments Involving Cell-Free (Circulating) Nucleic Acids. *Clinica Chimica Acta*, Vol. 363, No. 1-2, (January 2006), pp. 187-196, ISSN 0009-8981

van der Vaart, M. & Pretorius, P.J. (2008). A Method for Characterization of Total Circulating DNA. *Annals of New York Academy of Sciences*, Vol. 1137, (August 2008), pp. 92-97, ISSN 00778923

Veiko, N.N.; Bulycheva, N.V.; Roginko, O.A.; Veĭko, R.V.; Ershova, E.S.; Ozdoba, O.A.; Kuz'min, V.A.; Vinogradov, A.M.; Iudin, A.A.; Speranskii, A.I.  (2008). Ribosomal Repeat in the Cell Free DNA as a Marker for Cell Death. *Biomeditsinskaja Khimia*. Vol. 54, No. 1, (January-February2008), pp. 78-93, ISBN 0042-8809

Vlassov, V.V.; Laktionov, P.P.& Rykova, E.Y. (2010). Circulating Nucleic Acids as a Potential Source for Cancer Biomarkers. *Current Molecular Medicine*, Vol. 10, No. 2, (March 2010), pp. 142-165, ISSN 1566-5240

Wijeratne, S.; Butt, A.; Burns, S.; Sherwood, K.; Boyd, O.; Swaminathan, R. (2004). Cell-free plasma DNA as a Prognostic Marker in Intensive Treatment Unit Patients. *Annals of New York Academy of Sciences*, Vol. 1022, (June 2004), pp. 232-238, ISSN 00778923

**Technical Problems in Patients on Hemodialysis**

Edited by Prof. Maria Goretti Penido

This book provides an overview of technical aspects in treatment of hemodialysis patients. Authors have contributed their most interesting findings in dealing with hemodialysis from the aspect of the tools and techniques used.Each chapter has been thoroughly revised and updated so the readers are acquainted with the latest data and observations in the area, where several aspects are to be considered. The book is comprehensive and not limited to a partial discussion of hemodialysis. To accomplish this we are pleased to have been able to summarize state of the art knowledge in each chapter of the book.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

# INTECH
open science | open minds