Chronic renal failure is accompanied by endothelial activation and a large increase in microparticle numbers with reduced procoagulant capacity

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Abstract

Background. In patients with chronic renal failure (CRF), cardiovascular disease is the leading cause of increased morbidity and mortality. We hypothesized a role for endothelial activation and microparticle (MP) numbers and procoagulant activity in the pre-thrombotic state of these patients.

Methods. We analysed blood samples of 27 patients with CRF (8 chronic kidney disease Stage 4 (CKD4), 9 peritoneal dialysis (PD) and 10 haemodialysis (HD), samples taken before and after HD) and 10 controls. Degree and nature of endothelial activation were assessed by measuring mature von Willebrand factor (vWF) and vWF propeptide levels. Cellular MPs were characterized by flow cytometry and MP-specific thrombin generation (TG) measurements.

Results. CRF was accompanied by chronic (CKD4 and PD) or acute (HD) endothelial activation. Patients with CRF had substantially higher MP numbers than controls (median 9400 versus 4350 $\times 10^6$/L, $P = 0.001$), without significant differences between the treatment subgroups or between pre- and post-HD. The vast majority of MPs were platelet-derived. Of the minor populations, endothelial MPs and tissue factor-bearing MPs were more abundant in CRF. MPs were procoagulant, but the increase in numbers was not reflected in a proportional increase in MP-specific TG.

Conclusion. Renal failure is accompanied by endothelial activation of a different nature in CKD4 and PD patients compared to HD patients, and results in all subgroups in an increase of mainly platelet-derived MPs that appear to be less procoagulant than in other disease states, possibly because of the uraemic functional defect of their cellular source.

Keywords: chronic renal failure; microparticles; thrombin generation; tissue factor; von Willebrand factor

Introduction

Cardiovascular disease is the leading cause of mortality in patients with chronic renal failure (CRF), regardless of progression to end-stage renal disease [1, 2]. In fact, patients with chronic kidney disease (CKD) Stage 3 or 4 (glomerular filtration rate between 30 and 60 mL/min/1.73m$^2$) more often die of cardiovascular causes rather than progress to end-stage renal disease [3]. The development of cardiovascular disease in uraemic patients involves atherosclerosis, a complex process accompanied by endothelial dysfunction and inflammation. Moreover, patients with CRF have an increased risk of venous thromboembolism [4].

Circulating microparticles (MPs) are shed membrane vesicles of $<1.0 \mu m$ in diameter and are generated as a result of cellular apoptosis or activation in response to various stimuli [5]. MPs display specific cell surface proteins that indicate their cellular origin, e.g. platelets, leukocytes, endothelial cells or red blood cells. MPs expose phosphatidylserine on their outer membrane leaflet, which provides a suitable anionic phospholipid surface for assembly of the tenase and prothrombinase complexes [5, 6]. They may express tissue factor (TF) [7], the primary physiological initiator of coagulation. Such phosphatidylserine- and/or TF-bearing MPs contribute to thrombosis in different diseases. Moreover, MPs contribute to endothelial cell activation and dysfunction leading to vascular inflammation and development of atherosclerosis [8]. Indeed, in patients with cardiovascular disease, increased numbers of circulating MPs are observed [9, 10] and are associated with dysregulation of vascular tone [11, 12] and correlate with disease severity and clinical outcome [10].

In uraemic patients, elevated levels of circulating MPs have been detected as well [11, 13], and two possible mechanisms are described by which chronic renal disease may lead to elevated MPs levels. Firstly, in vitro high concentrations of uraemic toxins cause a rise in endothelial-derived.
Microparticles in CRF 1447

Blood samples were obtained from the arterial end of the fistula after 2 days of low-molecular weight heparin (LMWH) at the beginning of each HD dialysis session. Heparinization during dialysis was individually adjusted. The duration of dialysis was individually adjusted. The number of circulating MPs has been described to be increased in renal disease [11, 15, 17, 18]. Daniel et al. [15] described elevated numbers of granulocyte-derived MPs as a marker of inflammation in HD. MP circulating membrane-bound markers of coagulation, such as P-selectin and TF, have not as yet been described in renal disease. P-selectin-positive MPs reflect the source of platelet activation status and have been observed in increased numbers in myocardial infarction [19]. TF-bearing MPs are involved in arterial thrombosis [20], and TF is thought to play a major role in plaque thrombogenicity [21].

In the present study, we have made a first extensive inventory of the cellular and molecular spectrum of MPs in renal disease.

To gain more insight into the interplay between endothelial dysfunction and MP numbers and properties in CRF, we have assessed the activation status of the endothelium as reflected by plasma of mature von Willebrand Factor (vWF) and propeptide levels. The half-life of mature vWF measures four times the propeptide half-life, and due to this difference in half-life, their relative concentration is a distinctive indicator for ongoing chronic as opposed to acute endothelial activation [22]. Also, MP procoagulant capacity was assessed in an MP-dependent thrombin generation (TG) assay. Uniquely, this allowed us to eliminate pro- or anticoagulant effects of other patient plasma components since TG was performed by addition of isolated MPs to a normal human pooled plasma background.

Materials and methods

Patients

A cross-sectional study was performed in 27 patients with CRF (median age 54, range 22–80), including 10 patients with end-stage renal disease on HD, 9 patients with end-stage renal disease on peritoneal dialysis (PD), and 8 patients with CKD Stage 4 (CKD4, glomerular filtration rate between 15 and 30 mL/min/1.73 m<sup>2</sup>). Patients’ characteristics are listed in Table 1. Patients with diabetes or on coumarin derivatives were excluded. The average time of HD in the HD group was 2.6 years. The patients were dialysed three times a week with a low-flux polysulphone membrane (Fresenius Medical Care, Bad Homburg, Germany), and the duration of dialysis was individually adjusted to maintain an eKt/V > 1.3 per dialysis. All patients were dialysed in the same dialysis unit using the same dialysate system (Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands). Heparinization during dialysis session was performed in all patients with infusion of 2500–5000 IU of low-molecular weight heparin (LMWH) at the beginning of each HD session. All patients were dialysed through an arteriovenous fistula, and blood samples were obtained from the arterial end of the fistula after 2 days off dialysis, before and after HD. The PD group consisted of nine patients (three patients on continuous ambulatory PD and six on automated PD) and had an average time on dialysis of 2.2 ± 1.8 years. Blood samples of CKD4 and PD patients were obtained during the routine outpatient visits.

In addition, at the start of the study, we collected blood samples from 10 healthy individuals (median age 46, range 19–59), 6 men and 4 women who were not using oral contraceptives or hormonal therapy. In their blood samples, we analysed all parameters discussed here, except TG of isolated MPs added to human pooled plasma. As a healthy control group for this MP-specific TG experiment, designed and performed at a later point in time during the course of a study on MP in breast cancer patients (manuscript submitted), we used 20 healthy individuals (women, median age: 54 ± 12 years), not using oral contraceptives or hormonal therapy. We feel this is valid because the number of annexin V-positive MPs in this control group did not differ significantly from the first control group (median 6400 × 10<sup>6</sup>/L, P = 0.15), with a narrow distribution range. The mean fluorescence intensity (MFI) of MP-bound labelled annexin V was very similar in both control groups [MFI 416 in CTR (n = 20) and 438 in CTR (n = 10)], indicating similar MP procoagulant phospholipid exposure. Finally, in TG, no male/female differences have been found [23]. All patients and controls agreed to participate in the study following informed consent and this study was approved by the local medical ethical committee (Medisch Ethische Commissie, OLVG, Amsterdam).

Plasma markers of endothelial activation

Mature vWF and propeptide plasma levels were measured by enzyme-linked immunosorbent assay as described previously [24].

Blood collection, isolation and flow cytometric analysis of MPs

Blood samples were drawn with a 21-gauge needle after applying a light tourniquet. After discarding the first 4 mL, blood was collected into a 4.5-mL tube containing 3.2% trisodium citrate [Becton Dickinson (BD), Plymouth, UK]. Plasma was prepared within 20 min after blood collection by centrifugation for 20 min at room temperature at 1550 g, without a brake. Aliquots of plasma were snap frozen in liquid nitrogen and then stored at −80°C until use.

### Table 1. Patients’ characteristics

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<th>Polycystic kidney disease</th>
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<th>Albumin (g/L)</th>
<th>Creatinine (μmol/L)</th>
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<td>Venofer&lt;sup&gt;d&lt;/sup&gt; intravenous</td>
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</table>

<sup>a</sup>N.A., Not applicable.

<sup>b</sup>Angiotensin II receptor blockers.

<sup>c</sup>Angiotensin-converting enzyme.

<sup>d</sup>Ferri oxide saccharate (Venofer<sup>d</sup>) intravenous

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References

[11, 15, 17, 18] Daniel et al. [15] described elevated numbers of granulocyte-derived MPs as a marker of inflammation in HD. MP circulating membrane-bound markers of coagulation, such as P-selectin and TF, have not as yet been described in renal disease. P-selectin-positive MPs reflect the source of platelet activation status and have been observed in increased numbers in myocardial infarction [19]. TF-bearing MPs are involved in arterial thrombosis [20], and TF is thought to play a major role in plaque thrombogenicity [21].
MPs isolation was performed as previously described [25]. For flow cytometry analysis, MPs (5 µL) were diluted in 35 µL phosphate-buffered saline (PBS) containing 2.5 mM CaCl₂ (pH 7.4). Then, 5 µL Annexin V-APC from CalTag Laboratories (Burlingame, CA) and 5 µL fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- and/or peridinin chlorophyll protein complex (PerCP)-labelled cell-specific monoclonal antibodies (mAb) or isotype-matched control antibody were added. We used anti-CD41-FITC (V9, IgG1) from American Diagnostics (Stamford, CT); CD63-FITC (H5C6, IgG1), CD45-PerCP (H130, IgG 1, k), CD61-PE (VI-PL2, IgG2a), CD66b-FITC (80H3, IgG1), and CD4-PE (CLB-T4/2, IgG 1) and CD66acde-PE (CLB-gran/10, IH4Fc, IgG1) from DAKO (Glostrup, Denmark); CD144-FITC (BMS158FI, IgG1) from Bender MedSystems (Vienna, Austria), CD106-FITC (L11B1, IgG1) from Calbiochem (Darmstadt, Germany); CD54-PE (K662, IgG1), CD66b-FITC (80H3, IgG1, k) and CD62G-PE (CLB-Thromb/6, IgG1) from Immunotech (Marcelle, France); CD62E-FITC (HAE-1f, IgG1) from Kordia (Leiden, The Netherlands); CD4-PE (CLB-T4/2, IgG1) and CD66acde-PE (CLB-gran/10, IH4Fc, IgG1) from Sanquin (Amsterdam, The Netherlands). Labelled isotype controls IgG1, (X40) and IgG2a, (X39) were from BD (San Jose, CA) and IgG2a-PE (MCGB) from IQProducts ( Groningen, The Netherlands).

The mixtures were incubated in the dark for 30 min at room temperature. Subsequently, 760 µL PBS/calcium buffer was added. All samples were analysed for 1 min during which the flow cytometer analysed ~55 µL of the suspension. The samples were analysed in a FACS Calibur flow cytometer with CellQuest-pro software (BD). Forward scatter (FSC) and sideward scatter (SSC) were set to logarithmic gain. To distinguish MPs from events due to noise, MPs were identified on FSC, SSC and annexin V positivity. To identify MPs that bound cell-specific mAbs, MPs were incubated with identical concentrations of isotype-matched control antibodies to set the threshold. Some antibodies had higher background fluorescence than the isotype-matched control and with these antibodies, the threshold was set on the population MPs negative for the antibody. The number of MPs per litre plasma was calculated as previously described [25].

**TG measurements**

TG was measured by means of the calibrated automated thrombogram (CAT) method (Thrombinoscope BV, Maastricht, The Netherlands) [23]. TG was determined in the MP-rich plasma samples or in the supernatant on their membrane. The phenotypic characterization of MPs was of platelet origin, as determined by positivity for the platelet marker CD41 (CRF versus controls: 95 versus 94%). Reflecting the higher total number of MPs, the absolute number of platelet-derived MPs (CD41 positive) was significantly higher in CRF than in controls (9200 versus 4350 × 10⁶/L, P < 0.001; Table 3). No significant differences were observed between patient subgroups or between pre- and post-dialysis samples. The lowest median value was found in PD.

In most study objects >90% of circulating MPs-bound annexin-V, indicating the presence of phosphatidylserine on their membrane. The phosphatidylserine expression of MPs depicted in Figure 2 showed that the cellular subset composition of the MP population (i.e. of platelet-, leukocyte-, endothelial cell or erythrocyte origin) is similar between CRF and controls. The large majority of MPs in all groups was of platelet origin, as determined by positivity for the platelet marker CD41 (CRF versus controls: 95 versus 94%). Reflecting the higher total number of MPs, the absolute number of platelet-derived MPs (CD41 positive) was significantly higher in CRF than in controls (9200 versus 4100 × 10⁶/L, P < 0.001; Figure 2), again without differences between patient subgroups. Notably, the numbers of platelet-derived MPs did not correlate with urea levels.

**Results**

**Endothelial activation: mature vWF and propeptide**

We investigated the activation of endothelium by measuring mature vWF and its propeptide [22]. Table 2 shows that patients with CKD4 and PD had increased levels of mature vWF [median: CD44 63.6, PD 71.8 and controls 34.8 nM (ref range 26–49); P < 0.01 and P < 0.01], with similar (PD patients) or slightly elevated (CD44 patients) propeptide concentrations compared with controls [median: CD44 8.5, PD 8.0 and controls 5.2 nM (ref range 4.2–9.3)], indicating chronic endothelial activation (Figure 1). Mature vWF levels in HD patients were not elevated (median 39.5 nM). However, there was a moderate vWF increase during the dialysis process (median HD before 39.5 nM, HD after 46.0 nM, P = 0.48) with a disproportional rise in the propeptide levels, indicating acute endothelial activation (median HD before 4.7, HD after 8.0 nM, P = 0.04).

**Number and phenotypic characterization of MPs**

The total number of MPs was substantially increased in CRF patients compared with controls (9400 versus 4350 × 10⁶/L, P < 0.001; Table 3). No significant differences were observed between patient subgroups or between pre- and post-dialysis samples. The lowest median value was found in PD.

| Table 2. Levels of vWF antigen and propeptide presented as median and IQR |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| vWF antigen (nM) | 34.8 | 63.6* | 71.8* | 39.5 |
|                 | 28.1–37.0 | 42.8–78.0 | 62.3–106.4 | 30.6–54.8 |
| Propeptide (nM)  | 5.2    | 8.5  * | 8.0  | 4.7  |
|                 | 4.4–6.4 | 5.7–12.9 | 5.4–11.0 | 2.9–6.1 |

*P < 0.05 compared to controls.
**P < 0.05 compared to before dialysis session.
Co-expression of platelet (CD41) and activated platelet markers (CD62P and CD63) was increased in CRF (CD41, CD62P double-positive median 320 versus 140 × 10^6/L, P = 0.001; CD41, CD63 double-positive median 46 versus 8 × 10^6/L, P < 0.001). For CD62P-positive MPs, a relative increase was observed as well; in renal failure, they accounted for 3.6% of all MPs versus 2.0% in controls (Table 3, Figure 2). Although in PD patients the lowest numbers of MPs were observed, differences were not statistically significant in subgroups.

With regard to minor MP fractions, numbers of red blood cell-derived MPs (GlycophorinA+) were a priori hypothesized to be influenced by the HD process. However, similar numbers in all patient subgroups were shown and no significant increase upon HD was observed (Table 3, Figure 2). Although in PD patients the lowest numbers of MPs were observed, differences were not statistically significant in subgroups.

Endothelium-derived MPs, however, though relatively low, differed between groups with a higher number of MPs expressing CD144 in CKD4 and HD compared to controls, but not in PD. Whereas total numbers of leukocyte-derived MPs (CD45 positive) did not differ between CRF and controls, MPs positive for the granulocyte marker CD66e indeed were increased in CRF compared with controls (median: 14 versus 8 × 10^6/L, P = 0.02), and monocyte-derived MPs were observed in higher numbers in HD patients compared with controls (P = 0.005). All numbers of MPs observed in other subsets did not differ significantly from controls (Figure 2).

Finally, the number of TF-positive MPs, accounting for <1% of total MPs, was significantly higher in CRF as compared to controls. There were no differences between patient groups (Table 3) but relatively low values were observed in PD without any high outliers. TF was predominantly co-expressed with platelet marker CD41.

**TG experiments**

As a first experiment, TG was performed in MP-rich plasma. The reaction was initiated without exogenous TF and phospholipids added, thus rendering the assay MP sensitive. HD patient samples could not generate thrombin in this manner, probably due to the presence of trace amounts of LMWH before an HD session. Samples of CKD4 and PD patients did not differ from control samples.
Table 3. Number, cellular origin and composition of MPs in plasma (*10^6/L) in different patient groups and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls, n = 10</th>
<th>All CRF patients, n = 27</th>
<th>CKD4, n = 8</th>
<th>PD, n = 9</th>
<th>HD before, n = 10</th>
<th>HD after, n = 10</th>
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<tr>
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<td>4350 (3800–9400)</td>
<td>9400** (4000–25 500)</td>
<td>9150* (4000–13 100)</td>
<td>8500* (5000–25 500)</td>
<td>10 350* (4200–22 000)</td>
<td>13 350* (4000–22 200)</td>
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<tr>
<td>CD41 + MP</td>
<td>4100 (3500–9200)</td>
<td>9200** (3600–21 000)</td>
<td>9000* (3600–12 000)</td>
<td>7700* (4700–21 000)</td>
<td>10 230* (3800–19 000)</td>
<td>11 900* (3250–22 200)</td>
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<tr>
<td>CD62P + MP</td>
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<td>320** (70–720)</td>
<td>340** (220–720)</td>
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<td>410* (105–710)</td>
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<td>46** (8–190)</td>
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</table>

aData presented as median (range) or % of total (annexin V+) MPs.

*P < 0.05 compared with controls.

**P < 0.001 compared with controls.

Fig. 2. Size of circulating MP subpopulations in CRF patients and controls. Bar graph: number of MPs from specific cellular origin as defined by marker positivity in plasma from patient subgroups: CKD Stage 4 (CKD4), PD, HD and controls (CTR). Shown are the number of MPs positive for markers of platelets (CD41, CD61), activated platelets (CD62P, CD63), leukocytes (CD66e, CD14, CD20, CD66b, CD8, CD45, CD4, CD15), endothelial cells (CD62E, CD144, CD54, CD106), erythrocytes (GlycoA) and TF. Data presented as median. Inset: dotplot of the same data presented individually for the platelet marker CD41 and the marker for platelet activation CD62P. *P < 0.05 compared with controls.
in ETP [median: 1103 (524–1653) versus 1300 (596–1559) nM.min, P = 0.78] (Table 4).

In a following experiment, performed to verify the role of MPs in TG by excluding the role of the platelet plasma components, MPs were isolated and MPs and supernatant were added separately to normal pooled plasma and again tested for TG as described above. After removal of the MPs, the supernatant plasma did not show TG under these conditions (not shown), indicating that TG is MP dependent. Indeed, when isolated MPs were added to normal human pooled plasma, thrombin was generated and the ETP was lower in patient MPs than in control MPs (Table 4). Among the various patient subgroups, ETP values were the lowest in PD, without the differences between groups being statistically significant. The total number of annexin V-positive MPs (weakly) correlated with ETP (R = 0.43, P = 0.03; Figure 3).

When ETP values were expressed relative to MP numbers, MPs from CRF patients generated substantially less ETP than those from controls, or in other words, MPs in CRF possessed impaired thrombogenicity. MP phospholipid exposure is an important contributor to this property [26]. Interestingly, the MFI of MP-bound-labelled annexin V was significantly higher in the control group than in patients, which implies that MPs in CRF patients expose less procoagulant phospholipids [MFI 416 in CTR (n = 20) versus 305 in CRF, P < 0.001].

Discussion

This is the first study that in parallel assesses endothelial activation and characterizes MPs with an extensive panel of antibodies in different treatment groups of patients with CRF and studies MP-specific TG in these patients.

We observed that patients with CRF and an activated endothelial status had elevated levels of platelets, activated platelets and endothelial-derived MPs, irrespective of their dialysis modality. Other groups have described an absolute increase of platelet-derived MPs in CRF as well [11, 17]. This increase in MPs, which were procoagulant since they supported TG, did not result in a proportional increase in MP-dependent TG compared with controls. Apparently, the CRF MPs are not as potent thrombin generators as control MPs.

The endothelial activation parameters of mature vWF and propeptide provided evidence of an important difference between the CKD4 and PD groups, which showed a pattern compatible with chronic activation [22] and the HD group, which showed a pattern compatible with acute activation. This was inferred from the rise in vWF propeptide levels with an, apparently only moderate, mature vWF increase, observed during the HD session only. Previously, Tomura et al. [27] showed that patients on HD suffered from progressive endothelial cell damage and that the capacity of their endothelial cells for the release of vWF antigen gradually decreases in the months following initiation of HD. This may reflect the duration and intensity of endothelial challenge during HD, putatively leading to a relative vWF secretional exhaustion. However, it is shown here not to coincide with a significantly different MP profile or MP procoagulant capacity.

With further regard to endothelial activation in renal failure, not only plasma vWF levels but also endothelium-derived MPs are considered markers for endothelial dysfunction [11, 28] being predominantly produced by endothelial cells. In our study, endothelium-derived MPs (CD144+) accounted for ~1% of total MPs and as described before were thus a minor fraction [29]. Still, endothelium-derived MPs expressing CD144 were increased in CRF patients supporting the concept of ongoing endothelial activation. PD patients formed an exception in this regard: their CD144+ MP numbers did not differ from those in controls. This is in line with a recent study by Merino et al. [30] in which an increase was observed in endothelial damage-related CD14+ CD16+ cells in CKD-non-dialysis and HD patients but not in PD patients, suggesting a different pathway promoting the endothelial damage in PD.

As stated above, the observed increase in platelet- and endothelium-derived MPs in renal failure may be due to either uraemia or to the chronic vascular dysfunction and atherosclerosis that often accompanies CRF. The actual increase in MP numbers in the present study is substantial, with levels of MPs approximately two times higher in patients than in controls. (Pre)analytical conditions being similar, this indicates that metabolic dysregulation indeed plays a major role in the generation of high MP counts in renal failure. In agreement

Table 4. TG results for patient subgroups and controlsa

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CKD4</th>
<th>PD</th>
<th>HD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETP plasma (nM.min) (buffer)</td>
<td>1300 (596–1559)</td>
<td>1170 (857–1653)</td>
<td>1031 (524–1624)</td>
<td>N.D.</td>
<td>0.78</td>
</tr>
<tr>
<td>ETP MPs (nM.min) (buffer)</td>
<td>1029 (822–1234)</td>
<td>1263 (1017–1364)</td>
<td>1042 (852–1385)</td>
<td>1115 (932–1163)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

aResults are presented as median (range). P-value describes differences between the groups by Kruskal–Wallis test. Due to heparin remnants in plasma no ETP results in plasma are available for HD patients. N.D., Not determined.

Fig. 3. Correlation between number of annexin V-binding MPs and ETP (R = 0.43, P = 0.03) performed in human pooled plasma to which MPs of CRF patients were added. TG was initiated without exogenous TF and phospholipids.
with this conclusion, MP levels have been shown to rapidly decline after renal transplantation [31], when the renal function improves while the cardiovascular risk factors remain the same. In addition, renal clearance of MPs may be speculated to influence MP half-life in blood as urine is known to contain MPs [32]. If indeed MPs are removed from the circulation by renal clearance, the defective clearance in patients may also cause a rise in MP numbers.

Superimposed on uraemia, the HD process itself was a priori hypothesized to increase MP numbers as described previously [11, 15]. Perhaps surprisingly, we did not observe such an increase but counted similar levels of platelet-derived MPs before and after dialysis. This is in concordance with the results of Ando et al. [17] and Boulanger et al. [29]. A possible explanation for these findings is the use of different dialysis membranes. We used synthetic membranes, just as Boulanger et al. [29], while in the apparently conflicting studies [11, 15], cellulose membranes were used as well. The use of synthetic membranes might result in less platelet activation although attachment of MPs to the membrane during dialysis cannot be excluded.

We show an absolute increase of MPs carrying markers of platelet activation (CD62P and CD63) [19] in CRF that may contribute to an increased risk of vascular disease. CD62P, or P-selectin, is an important player in one of the cellular pathways for the initiation of blood coagulation. P-selectin-positive platelets are known to induce leukocyte-derived MPs to express TF, which can induce clot formation [33, 34]. P-selectin expression by MPs is likely to have the same effect. In support of this, we did find higher numbers of leukocyte-derived MPs and TF-bearing MPs in patients with CRF, although the account of both subsets was low (<1%).

The procoagulant potential of the CRF MPs was confirmed in TG experiments. MP-rich plasma samples from CKD4 and PD patients could generate thrombin under MP-dependent (exogenous TF and phospholipid free) conditions. Despite the higher MP numbers in these samples, the resulting ETPs were not different from those in controls. This suggested either less potent procoagulant properties of the CRF MPs and/or a negative influence of patient plasma components (inhibition, coagulation factor depletion). TG experiments with isolated control- and CRF MPs added to normal pooled MP-free plasma provided evidence for the former mechanism. The higher number of MPs in the isolates of CRF patients resulted in a higher, but not proportionally higher with respect to MP numbers, TG in the normal plasma background. This was not due to an overload of MPs since in this MP number range for CRF samples a dose–response relationship with ET values was apparent (no plateau was reached yet). These data suggest that MPs in CRF patients indeed are procoagulant but at the same time points to a qualitative difference in MP properties between CRF patients and controls. Herewith, we provide another example of disease-related MP populations with disease-specific procoagulant potential. In CRF, it refers to a decreased potential, whereas in a previous study, we identified myeloblast-derived MPs in Acute Myeloid Leukemia patients that were more intrinsically procoagulant than control MPs [35]. With regard to the relatively poor TG capacity of the CRF MPs, it is of interest to note that their main cellular source (platelets) is functionally challenged by the kidney failure (uraemic thrombopathy). For example, uraemic platelets have been reported to show diminished adhesion and aggregation [36]. It may therefore be speculated that thrombopathic platelets in uraemia generate ‘thrombopathic’ MPs being only moderately procoagulant. Indeed, we found that MPs in CRF showed a diminished exposure of phosphatidylserine, which is expected to result in a lower ETP [37].

It will be of interest to further study the role of plasma components in the net similar MP-driven TG in the control and patient MP-rich plasma samples. Since apparently the role of MPs in cardiovascular complications in CRF does not so much derive from an increase in MP-driven plasmatic coagulability, we speculate that their function is altered regarding the cellular processes leading to endothelial damage and/or the recruitment of procoagulant factors in vivo. This is in line with a recent study by Terrisse et al. [38] showing that endothelial cells internalized MPs, which resulted in enhanced platelet/endothelium interactions, involving vWF and P-selectin. Indeed, in CRF, atherosclerosis is the main vascular problem, not venous thrombosis in which hypercoagulability would be expected.

In conclusion, CRF is accompanied by endothelial activation of a different nature in CKD4 and PD patients versus HD patients but results in all subgroups in an increase (without increment during HD) of mainly platelet-derived MPs with minor endothelial marker—and TF-positive fractions. Although presence of these MPs in higher numbers may explain an increased prothrombotic state, these MPs appear to be less procoagulant than in other disease states, possibly because of a functional defect of their cellular source due to chronic uraemia. In spite of this, these MPs may still be important messengers at the cellular level in the development of atherosclerosis. Further studies are required to investigate these functional properties of MPs in CRF.

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Microparticles in CRF


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