symmetrical arms (Figure 1), starch causes irregular ‘Maltese crosses’, with rather asymmetrical arms, as can be easily seen in figure 2 of the Chaudry and Sedlacek paper, in our Figure 2, as well as in other sources [2,3].

Therefore, starch granules are distinguishable from fatty compounds and 2,8DHA crystals by polarized light.

Starch granules also differ from the other two particles under bright field and phase contrast microscopy: starch granules are colourless, are variable in size, a roundish to polygonal shape, with a nucleus-like centre [2,3]; lipid droplets are round and translucent without any internal structure; 2,8DHA crystals are round, have a reddish-brown colour, central spicules and dark outlines [4].

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Note: Dr Chaudhry et al. were invited to provide a reply, but did not answer this invitation.

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The influence of 24 h urine sampling on the evaluation of renal dysfunction

Sir,
Renal dysfunction and the treatment of renal disease depend on glomerular filtration rate (GFR) and proteinuria (Upr). Current guidelines recommend the use of creatinine–based estimation equations to predict GFR in patients with chronic kidney disease (CKD) [1]. The accuracy of these equations has been questioned, in patients with altered body composition, in proportion to muscle mass (i.e. male/female) [2]. Even the proposed estimation of GFR using cystatin C is not independent of body composition [3]. For these reasons, 24 h urinary collection (U24h) remains the gold standard for the estimation of creatinine clearance (Ccr), at least for the initial evaluation in every day clinical practice. The same collection can also be used for the estimation of the degree of Upr, although the urinary protein/creatinine ratio determined on a random urine specimen is considered a practical and useful guide [4]. The U24h is a colloid solution which contains uncharged, positive and negative charged components. Although the most common sources of error when estimating the Ccr, are the loss of a portion of U24h collection and U24h retention, the U24h must be well-shaken, since the long (24 h)
Table 1. The degree of proteinuria (Upr) and creatinine clearance (Ccr) and other measured parameters in the five groups of the urinary samples

<table>
<thead>
<tr>
<th>Groups</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 25)</td>
<td>II (n = 25)</td>
</tr>
<tr>
<td>Upr (g/24 h)</td>
<td>BS sample</td>
</tr>
<tr>
<td></td>
<td>AS sample</td>
</tr>
<tr>
<td></td>
<td>Difference (%)</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>BS sample</td>
</tr>
<tr>
<td></td>
<td>AS sample</td>
</tr>
<tr>
<td></td>
<td>Difference (%)</td>
</tr>
<tr>
<td>Urea (g/24 h)</td>
<td>BS sample</td>
</tr>
<tr>
<td></td>
<td>AS sample</td>
</tr>
<tr>
<td></td>
<td>Difference (%)</td>
</tr>
<tr>
<td>Phosphorus (mg/24 h)</td>
<td>BS sample</td>
</tr>
<tr>
<td></td>
<td>AS sample</td>
</tr>
<tr>
<td></td>
<td>Difference (%)</td>
</tr>
<tr>
<td>Uric acid (mg/24 h)</td>
<td>BS sample</td>
</tr>
<tr>
<td></td>
<td>AS sample</td>
</tr>
<tr>
<td></td>
<td>Difference (%)</td>
</tr>
</tbody>
</table>

*P < 0.05 between AS and BS sample in each group.

Molecular weight: creatinine 113.1, urea 60.1, uric acid 168.1, phosphorus 96.0, proteins 14000–150000.

Charge: urea/creatine/uric acid uncharged, phosphorus negative, proteins positive/negative.

The aim of the present study was to investigate the possible difference in the results when the sample was taken before or after the U24h collection had been shaken.

One hundred and five patients (57 m, 48 f), aged 20–80 years, with CKD stages 1–3 (MDRD formula) were included in the study. Patients were instructed to begin the U24 h collection (before shaking—BS) while the second after the U24 h collection had been shaken (AS). Serum and urinary creatinine concentration were determined using the Jaffe method. The urine collections were classified in 5 groups according to the degree of Upr, as estimated via the BS sample [group I (n = 25) Upr < 0.15 g, group II (n = 25) 0.15 ≤ Upr < 1.0 g, group III (n = 20) 1.0 g ≤ Upr < 2.0 g, group IV (n = 20) 2.0 g ≤ Upr < 3.5 g and group V (n = 15) Upr ≥ 3.5 g].

All data are reported as mean ± SD. A student’s t-test was used for the comparison between the BS and AS samples. A value of P < 0.05 was considered significant.

We did not find any statistical significant difference in groups I and II in Upr and Ccr, while in groups III–V we observed a statistically significant difference (P < 0.05) between the BS and AS samples for both Ccr and Upr (Table 1). No significant difference was observed in the samples for the other measured parameters. The Upr in all samples—shaken and unshaken—are represented in the Figure 1.

The results of our study demonstrated that the procedure of U24h sampling influences the degree of Upr and Ccr.
the precipitation (saturation point) of the diluting elements. Thus, taking samples from the upper part of the U_{24h} (un shaken sample) may give wrong values for the degree of Upr and Ccr. In CKD patients, the quantification of Upr excretion is the most important test for the initial diagnosis and follow-up of patients with glomerulopathies. The response to immunosuppressive drugs or to other therapeutic interventions is defined by the degree of Upr reduction in patients with nephrotic and non-nephrotic types of Upr. An estimation of the degree of Upr which is based on BS samples falsely leads to the assumption that a partial remission is evident, whereas the real degree of Upr is actually higher. On the other hand, the value of Ccr guides the nephrologist to adjust the patient’s protein intake, and avoid potentially nephrotoxic drugs or interfering with sodium and potassium homeostasis.

In conclusion, it seems that the procedure of U_{24h} sampling can influence the estimation of renal dysfunction based on Ccr and the degree of Upr. For that reason, correct U_{24h} collection and sampling examination after shaking are the necessary procedures for the correct initial estimation of renal dysfunction.

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**A case of post-allogeneic haematopoietic stem cell transplantation membranous nephropathy**

Sir,

We read with special interest the paper by Terrier et al. [1], regarding the occurrence of membranous nephropathy (MN) after allogeneic haematopoietic stem cell transplantation (HSCT). The authors described five patients who presented with membranous nephropathy, with history and active manifestations of chronic graft-vs-host disease (GVHD) at MN diagnosis, suggesting that chronic GVHD might contribute to the development of MN after allogeneic HSCT. We have, however, encountered a patient who showed nephrotic syndrome due to MN after allogeneic HSCT without any history or manifestations of GVHD.

A 38-year-old Japanese man with acute myeloid leukaemia (AML, M4 with eosinophilia) in second remission underwent an allogeneic HSCT from an HLA-identical donor. Total body irradiation and cyclophosphamide were used as conditioning therapy, a short-time methotrexate and cyclosporine (CsA) as GVHD prophylaxis. As he did not show any evidence of acute or chronic GVHD, CsA was tapered and withdrawn after 10 months. Several months later, hypoalbuminaemia developed, and marked proteinuria was found at 20 months after HSCT; he was thus referred to our department. On admission he presented with nephrotic syndrome, with daily protein excretion of 13.1 g. serum albumin of 2.4 g/dl and reduced renal function (serum creatinine, 0.79 mg/dl and glomerular filtration rate, 44.5 ml/min). He had never presented any clinical or laboratory manifestations of chronic GVHD (e.g. skin lesions, gastrointestinal manifestations or liver function abnormalities), nor signs of recurrence of leukaemia. Serological studies including complements, anti-nuclear antibodies and hepatitis B/C, yielded negative/normal results. A perecutaneous renal biopsy revealed normocellular glomeruli with mild capillary wall thickening and marked renal tubular atrophy with interstitial fibrosis. Immunofluorescent study revealed granular immune deposition for IgG on the capillary wall, but no deposition of IgA, IgM, C3 C4 or Clq. Electron microscopy showed small and discreet electron-dense subepithelial deposits without basement membrane reaction, indicating early stage MN. As we suspected, his renal interstitial lesions might be evoked by CsA nephrotoxicity, we initiated corticosteroid therapy (prednisolone, 40 mg/day).

As described by Terrier et al. [1], several reviews of the literature reveal a close temporal relationship between the development of MN shortly after cessation of immunosuppressants and the diagnosis/presentation of chronic GVHD in allogeneic HSCT patients [1–5]. Further, there are few reports describing MN patients without acute or chronic GVHD after allogeneic HSCT, indicating that MN is thought to be a renal manifestation of GVHD. As his nephrotic syndrome developed after the cessation of CsA, MN is thought to be the only manifestation of chronic GVHD. Our patient is, however, characterized by the presence of chronic GVHD (e.g. skin lesions, cutaneous manifestations of chronic graft-vs-host disease (GVHD); thus, there is a possibility that de novo MN might have occurred in our case. Our case merits presentation because further accumulation of clinical studies including case reports is necessary, to confirm whether MN is a real manifestation of GVHD after allogeneic HSCT or merely a coincidence.

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