CD14$^+$CD16$^+$ monocytes and cardiovascular outcome in patients with chronic kidney disease

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Aims Patients with chronic kidney disease (CKD) pose a worldwide growing burden to health care systems due to accelerated atherosclerosis and subsequent high cardiovascular (CV) morbidity. Atherogenesis is prominently driven by monocytes and monocyte-derived macrophages. The expression of CD14 and CD16 characterizes three monocyte subsets: CD14$^+$CD16$^-$, CD14$^+$CD16$^+$, and CD14$^{++}$CD16$^+$ cells; the latter two are often denoted as ‘proinflammatory’ CD16$^+$ monocytes. Despite an association between CD16$^+$ monocyte counts and higher CV risk in cross-sectional cohorts, the prognostic impact of elevated CD16$^+$ monocyte counts is poorly understood.

Methods and results We assessed monocyte heterogeneity using flow cytometry in 119 patients with non-dialysis CKD, who were prospectively followed for a median of 4.9 (inter-quartile range 4.8–5.0) years for the occurrence of CV events. In addition, we assessed expression of chemokine receptors on monocyte subsets. CD14$^+$CD16$^+$ monocyte were independently associated with CV events [hazard ratio (for an increase of 10 cells/μL) 1.26 (confidence interval: 1.04–1.52; P = 0.018)] after adjustment for variables that significantly affected CD14$^+$CD16$^+$ cell counts at baseline. Across the spectrum of CKD, CD14$^+$CD16$^+$ monocytes selectively expressed CCR5.

Conclusion We found that CD14$^+$CD16$^+$ monocytes were independently associated with CV events in non-dialysis CKD patients. Our results support the notion that CD16$^+$ monocytes rather than CD16$^-$ monocytes are involved in human atherosclerosis.

Keywords Monocyte heterogeneity • Cardiovascular outcome • Chronic kidney disease • Cardio-renal syndrome

Introduction

Monocytes and monocyte-derived macrophages are at the centre stage of the innate immune system, fulfilling important tasks in host-defence, immunoregulation, tissue repair, and regeneration. Nonetheless, monocyte biology in health and disease is still poorly understood, and puzzling findings remain, such as the missing coherent association between monocyte counts and cardiovascular (CV) disease in large epidemiological studies. This is somehow counterintuitive, as monocytes and macrophages are well-established key players in atherosclerosis.

A possible explanation might be that human monocytes were considered to be a homogenous leucocyte subpopulation until 1989, when human monocyte heterogeneity was reported for the first time. Presently, three human monocyte subsets are defined by the differential expression of the LPS receptor CD14 and the FcγII receptor CD16, which are CD14$^+$CD16$^-$ cells, CD14$^+$CD16$^+$ cells, and CD14$^{++}$/CD16$^+$ cells. In earlier studies, the latter two subsets are summarized as CD16$^+$ monocytes, which account for 10–20% of all circulating monocytes. As opposed to classical CD14$^+$CD16$^-$ monocytes, CD16$^+$ monocyte counts are elevated in numerous inflammatory conditions, including end-stage renal disease. Therefore, CD16$^+$ monocytes have traditionally been termed ‘proinflammatory’ monocytes.

In line, dialysis patients with high CD14$^+$CD16$^+$ monocyte counts are at increased risk for future CV events. Obviously, dialysis patients are a highly selected population, so that findings from this population cannot be transferred to other patient groups. Therefore, we see a pressing need for further experimental and clinical research in human monocyte heterogeneity to clarify...
the significance of the respective monocyte subsets for human path-
ology and to test whether the predictive role of CD14$^{++}$CD16$^+$
monocytes in dialysis patients for CV events holds true in broader
patient groups.

In the present study, we demonstrate that CD14$^{++}$CD16$^+$
monocytes are independently associated with CV events in
patients with non-dialysis chronic kidney disease (CKD), even
though CD16$^+$ monocyte counts in CKD patients are close to
the range observed in healthy individuals and considerably lower
than in patients on haemodialysis.

**Methods**

**Study population**

In a prospective cohort study on monocyte heterogeneity and CV
outcome in CKD, 152 stable ambulatory patients with CKD
K/DOQI 1–5 not receiving renal replacement therapy were screened.
In all patients, comorbidity was determined by standardized interviews
and by review of medical documentation. Thirty-one patients were
excluded from the analysis as they were on immunosuppressive treat-
ment, and in two patients, determination of monocyte subsets failed
due to lost blood samples, leaving 119 patients in the study. In this
cohort, CKD was due to diabetic nephropathy ($n=26$), glomerulone-
phritis ($n=20$), interstitial nephritis ($n=14$), nephrosclerosis ($n=
13$), autosomal dominant polycystic kidney disease ($n=8$), obstructive
nephropathy ($n=4$), other primary renal diseases ($n=20$), and
unknown conditions ($n=14$).

Prevalent CV disease was defined as a history of myocardial infarction,
coronary artery angioplasty/stenting/bypass surgery, stroke, carotid
endarterectomy/stenting, non-traumatic lower extremity amputation,
or lower limb artery bypass surgery/angioplasty/stenting. Diabetes mel-
litus was diagnosed if a patient had a history of diabetes mellitus, a spon-
taneous plasma glucose level of $>200$ mg/dL, self-reported diabetes
mellitus, and/or received hypoglycaemic treatment. Patients were cate-
gorized as active smokers if they were current smokers or had stopped
smoking $<1$ month before entry into the study. Systolic and diastolic
blood pressures (BP sys and BP dia) were measured in a supine position.
Pulse pressure was calculated as BP sys $- BP$ dia.

**Table 1** Baseline characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Overall ($n=119$)</th>
<th>No event ($n=72$)</th>
<th>Event ($n=47$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66 (54–71)</td>
<td>63 (50–70)</td>
<td>70 (64–76)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Women (%)</td>
<td>54 (45)</td>
<td>35 (49)</td>
<td>19 (40)</td>
<td>0.479</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>11 (9)</td>
<td>9 (13)</td>
<td>2 (4)</td>
<td>0.143</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>42 (35)</td>
<td>17 (24)</td>
<td>25 (53)</td>
<td>0.001</td>
</tr>
<tr>
<td>History of CVD (%)</td>
<td>41 (34)</td>
<td>18 (25)</td>
<td>23 (49)</td>
<td>0.015</td>
</tr>
<tr>
<td>K/DOQI stage 1 (%)</td>
<td>2 (2)</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td>0.008</td>
</tr>
<tr>
<td>K/DOQI stage 2 (%)</td>
<td>16 (13)</td>
<td>13 (18)</td>
<td>3 (6)</td>
<td></td>
</tr>
<tr>
<td>K/DOQI stage 3 (%)</td>
<td>45 (38)</td>
<td>33 (46)</td>
<td>12 (26)</td>
<td></td>
</tr>
<tr>
<td>K/DOQI stage 4 (%)</td>
<td>28 (23)</td>
<td>13 (18)</td>
<td>15 (32)</td>
<td></td>
</tr>
<tr>
<td>K/DOQI stage 5 (%)</td>
<td>28 (23)</td>
<td>11 (15)</td>
<td>17 (36)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>201 (163–231)</td>
<td>208 (163–233)</td>
<td>195 (161–228)</td>
<td>0.602</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>50 (40–59)</td>
<td>52 (40–61)</td>
<td>48 (41–53)</td>
<td>0.318</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>29 (26–33)</td>
<td>29 (26–33)</td>
<td>29 (25–33)</td>
<td>0.859</td>
</tr>
<tr>
<td>Plasma calcium (mmol/L)</td>
<td>2.4 (2.3–2.4)</td>
<td>2.4 (2.2–2.4)</td>
<td>2.4 (2.3–2.4)</td>
<td>0.327</td>
</tr>
<tr>
<td>Plasma phosphorus (mg/dL)</td>
<td>3.6 (3.1–4.4)</td>
<td>3.5 (3.0–4.2)</td>
<td>3.9 (3.3–5.0)</td>
<td>0.031</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>3.2 (2.9–7.6)</td>
<td>2.9 (2.9–6.4)</td>
<td>4.3 (2.9–13.2)</td>
<td>0.117</td>
</tr>
<tr>
<td>Plasma homocystein (μmol/L)</td>
<td>15 (12–21)</td>
<td>14 (11–20)</td>
<td>18 (13–27)</td>
<td>0.001</td>
</tr>
<tr>
<td>Proteinuria (g/g creatinine)</td>
<td>0.4 (0.0–2.2)</td>
<td>0.2 (0.0–1.4)</td>
<td>1.7 (0.0–3.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>170 (150–185)</td>
<td>165 (146–184)</td>
<td>175 (155–195)</td>
<td>0.061</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>95 (85–110)</td>
<td>95 (86–109)</td>
<td>95 (80–110)</td>
<td>0.752</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>70 (60–85)</td>
<td>68 (55–85)</td>
<td>75 (65–85)</td>
<td>0.011</td>
</tr>
<tr>
<td>Antiplatlet therapy (%)</td>
<td>41 (35)</td>
<td>40 (28)</td>
<td>21 (45)</td>
<td>0.049</td>
</tr>
<tr>
<td>Beta-blockers (%)</td>
<td>64 (55)</td>
<td>36 (51)</td>
<td>28 (61)</td>
<td>0.447</td>
</tr>
<tr>
<td>Angiotensin-receptor blockers (%)</td>
<td>66 (57)</td>
<td>38 (54)</td>
<td>28 (61)</td>
<td>0.442</td>
</tr>
<tr>
<td>ACE-inhibitors (%)</td>
<td>44 (38)</td>
<td>28 (40)</td>
<td>16 (35)</td>
<td>0.440</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>41 (35)</td>
<td>23 (32)</td>
<td>18 (38)</td>
<td>0.695</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>13.0 (11.6–14.3)</td>
<td>13.3 (12.4–14.4)</td>
<td>12.0 (11.2–14.2)</td>
<td>0.014</td>
</tr>
<tr>
<td>Platelets ($\times 10^9$ cells/μL)</td>
<td>230 (188–268)</td>
<td>222 (183–261)</td>
<td>235 (189–268)</td>
<td>0.715</td>
</tr>
<tr>
<td>Leucocytes (cells/μL)</td>
<td>6800 (5600–7800)</td>
<td>6550 (5400–7550)</td>
<td>7000 (5800–8400)</td>
<td>0.257</td>
</tr>
<tr>
<td>Neutrophils (cells/μL)</td>
<td>4030 (3192–5037)</td>
<td>3838 (3188–4676)</td>
<td>4488 (3216–5548)</td>
<td>0.150</td>
</tr>
<tr>
<td>Lymphocytes (cells/μL)</td>
<td>1606 (1323–2072)</td>
<td>1628 (1358–2166)</td>
<td>1584 (1232–1974)</td>
<td>0.236</td>
</tr>
</tbody>
</table>

Variables are presented as percentage, or as median with inter-quartile range, as appropriate.
Informed consent was obtained from all patients, and the study design was approved by the local Ethics Committee. All participants were followed from the baseline examination in 2004 until death or until 31 July 2009. One patient was lost to follow-up. The pre-specified combined clinical endpoint was the first occurrence of a CV event (defined as myocardial infarction, coronary artery angioplasty/stenting/bypass surgery, stroke with symptoms lasting >24 h, carotid endarterectomy/stenting, non-traumatic lower extremity amputation, lower limb artery bypass surgery/angioplasty/stenting, or death). All-cause mortality was assessed as a secondary endpoint.

To assess whether the reported shift in monocyte subset counts in CKD patients occurs before or after the onset of dialysis treatment, we compared monocyte subset counts among 39 controls with intact renal function, 39 patients suffering from advanced CKD (stages 4/5, not yet undergoing renal replacement therapy), and 39 dialysis patients.

These groups were matched for age (±5 years), gender, prevalent CV disease, and diabetes mellitus, as defined above.

**Laboratory methods**

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), calcium, phosphorus, albumin, and C-reactive protein were measured using standard techniques. Glomerular filtration rate was estimated (eGFR) using the MDRD study equation 4. Differential blood counts were determined with automated cell counters.

Via flow cytometry, monocyte subsets were identified according to our previously published standard staining and gating strategy in a whole blood assay using 100 μL of heparin anticoagulated blood. Cells were stained with monoclonal antibodies—anti-CD86 (HA5.2B7, Beckman-Coulter, Krefeld, Germany), anti--CD16 (3G8, Invitrogen, Hamburg, Germany), anti-CD14 (M09, BD Biosciences, Heidelberg, Germany), and analysed by flow cytometry (FACSCalibur, BD Biosciences) using the Cell Quest software.

Monocytes were gated in a SSC/CD86 dotplot, identifying monocytes as CD86 cells with monocyte scatter properties. Subpopulations of CD14++CD16−, CD14++CD16+, and CD14−CD16+ monocytes were distinguished by their surface expression pattern of the LPS receptor CD14 and the Fc\III receptor CD16. Using these basic panels, monocyte subpopulations were further examined for the expression of the chemokine receptors CCR2, CCR5, and CX3CR1 in 30 patients, equally distributed across CKD stages 2–4. The following monoclonal antibodies were used: anti-CCR2 (48607, BD Biosciences), anti-CCR5 (2D7, BD Biosciences), and anti-CX3CR1 (2A9-4, Biozol, Eching, Germany). Flow cytometrical data were measured as median fluorescence intensity and standardized against coated fluorescent particles (SPHERO\textsuperscript{TM}, BD Biosciences).

**Statistical analysis**

Categorical variables are presented as percentage of patients, and compared by Fisher’s exact test. Continuous data are expressed as medians with inter-quartile range (IQR) and compared by
Mann–Whitney test (for two independent samples) or by Friedman test (for paired samples), as appropriate. Correlation coefficients were calculated by Spearman test. Kaplan–Meier survival curves were calculated, and event-free survival (i.e. time until first CV event as defined above) as well as overall survival (i.e. time until death of any cause) were compared by log-rank test. Cox proportional-hazards models were calculated to examine the relationship of monocyte subset cell counts with event-free survival after adjustment for variables that were associated with CD14\(^{++}\)CD16\(^{+}\) monocyte counts at baseline, and for eGFR.

Data management and statistical analysis were performed with SPSS 17.0. The level of significance was set at \(P \leq 0.05\).

**Results**

**Baseline characteristic**

The baseline characteristics of all 119 study participants are shown in Table 1. Forty-seven patients (39.5%) experienced a CV event before 31 July 2009. One patient was lost after a follow-up period of 1.8 years; the remaining 71 patients have been followed for a median of 4.9 (IQR 4.8–5.0) years. As expected, those patients who had a CV event were older, had a higher prevalence of diabetes mellitus at baseline, higher pulse pressure measurements, and more advanced CKD with lower eGFR, higher proteinuria and higher plasma phosphate levels (Table 1).

At baseline, eGFR correlated neither with total monocyte counts \((r = 0.048, \ P = 0.601)\) nor with monocyte subpopulation counts \((CD14^{++}CD16^{-}: r = 0.012, \ P = 0.895; CD14^{++}CD16^{+}: r = 0.117, \ P = 0.205; CD14^{+}(+)CD16^{+}: r = 0.094, \ P = 0.310)\). Similarly, total monocytes and monocyte subset counts were not significantly associated with BP sys, pulse pressure, serum phosphate, and proteinuria (data not shown). Conversely, CD14\(^{++}\)CD16\(^{+}\) and CD14\(^{+}(+)\)CD16\(^{+}\) monocytes were significantly correlated with age \((r = 0.187, \ P = 0.042\) and \(r = 0.206, \ P = 0.025\), respectively), whereas total monocytes and CD14\(^{++}\)CD16\(^{-}\) were not. Interestingly, CD14\(^{++}\)CD16\(^{+}\) monocytes were the only monocyte subset to be significantly correlated with C-reactive protein \((r = 0.253, \ P = 0.006)\), whereas CD14\(^{+}(+)\)CD16\(^{+}\) monocytes were significantly associated with serum homocystein levels \((r = 0.201, \ P = 0.038)\).

At study enrolment, CD14\(^{++}\)CD16\(^{+}\) monocyte counts were significantly elevated among patients with prevalent diabetes mellitus [diabetics: median 32 (IQR 21–39); non-diabetics: median 22 (IQR 17–34) cells/\(\mu\)L, \(P = 0.009\)], and prevalent CV disease [CV disease: median 27 (IQR 21–37); no CV disease median 23 (17–36) cells/\(\mu\)L, \(P = 0.046\)], whereas patients on statin treatment had higher counts of CD14\(^{+}(+)\)CD16\(^{+}\) monocytes [statin intake: median 75 (IQR 56–111); no statin intake: median 61 (IQR 47–86) cells/\(\mu\)L; \(P = 0.028\)], but not of CD14\(^{++}\)CD16\(^{-}\).
CD14\(^{++}\)CD16\(^{+}\) monocytes, or total monocytes, respectively. Intake of antiplatelet agents, angiotensin-converting enzyme (ACE)-inhibitors, angiotensin-receptor blockers, and beta-blockers was not associated with differences in monocyte (subset) counts.

**CD14\(^{++}\)CD16\(^{+}\) monocytes and their relation to cardiovascular outcome and mortality**

Patients who experienced a CV event during follow-up had higher CD14\(^{++}\)CD16\(^{+}\) monocyte counts compared with patients without an event, whereas counts of total monocytes, CD14\(^{++}\)CD16\(^{-}\), and CD14\(^{+}\)CD16\(^{-}\)monocytes did not differ significantly. When stratifying patients by their CD14\(^{++}\)CD16\(^{+}\) monocyte counts, patients in the highest tertile had the shortest event-free survival (Figure 1). Likewise, these patients had the shortest overall survival (Figure 2). The prognostic impact of this monocyte subset is even strengthened when stratifying patients by their percentage of CD14\(^{++}\)CD16\(^{+}\) monocytes (defined as % of all circulating monocytes) instead of absolute counts of CD14\(^{++}\)CD16\(^{+}\) monocytes (see Supplementary material online, Figures S1 and S2).

In contrast, tertiles of CD14\(^{++}\)CD16\(^{-}\) (Figure 3) and CD14\(^{+}\)CD16\(^{-}\) monocytes (Figure 4) did not predict survival in our patient cohort. In Cox regression analysis, CD14\(^{++}\)CD16\(^{+}\) monocyte counts remained significantly associated with event-free survival after adjustment for variables that were correlated with CD14\(^{++}\)CD16\(^{+}\) monocyte counts at baseline (age, diabetes mellitus, prevalent CV disease, and C-reactive protein), and for eGFR (Table 2).

**Chemokine receptor expression on monocyte subsets**

The chemokine receptors CCR2, CCR5, and CX3CR1 are relevant in subset-specific extravasation of monocytes in atherosclerotic plaques.\(^{20}\) Therefore, we analysed monocyte surface expression pattern of these chemokines receptors in 30 CKD patients. Flow cytometry confirmed monocytic subset-specific expression of chemokine receptors across different stages of CKD. Although CD14\(^{++}\)CD16\(^{-}\) monocytes expressed highest levels of CCR2, and CD14\(^{+}\)CD16\(^{-}\)monocytes express highest levels of CX3CR1, CD14\(^{++}\)CD16\(^{+}\) monocytes are characterized by selective expression of CCR5 and coexpression of both CCR2 and CX3CR1 irrespective of kidney function (Figure 5).

**Comparison of chronic kidney disease patients to subjects with normal renal function and haemodialysis patients**

As we observed no significant increase in monocyte subset counts with declining renal function—contrasting the known expansion of
CD16$^+$ monocytes and CV outcome in CKD

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CD16$^+$ monocytes in haemodialysis patients$^{16}$—we compared 39 subjects with normal renal function, 39 patients with CKD (not receiving renal replacement therapy), and 39 haemodialysis patients matched for age, gender, prevalent CV disease, and diabetes mellitus.

In each group, 18 out of 39 (46%) patients were male, 22 patients (56%) were diabetics, and 14 patients (36%) had a history of CV disease. Counts of CD16$^+$ monocytes significantly differed among the three groups, with lower counts of CD14$^+$CD16$^+$ and CD14$^{(+)}$CD16$^+$ monocytes in subjects with normal renal function and CKD patients compared with haemodialysis patients (Figures 6 and 7).

**Discussion**

Research interest in monocyte heterogeneity gained strong momentum in the last decade, as a subset-specific contribution of monocytes to atherogenesis has been postulated.$^{21}$ However, human monocyte heterogeneity and its relation to human CV disease are still poorly understood.

We have previously reported on the relationship of CD16$^+$ monocytes and CV events in dialysis patients. In these studies, baseline CD14$^+$CD16$^+$ monocyte counts$^{18}$ and haemodialysis-induced CD16$^+$ monocyte kinetics independently predicted CV outcome.$^{22}$ Of note, dialysis patients are a highly selected population, and epidemiological data from these patients cannot be extrapolated to other individuals. Specifically, dialysis patients experience a tremendous CV event rate. At the same time, they show a notable elevation of CD16$^+$ monocyte counts compared with patients with intact renal function.$^{16}$

It has been fully unknown whether this shift in monocyte subpopulations towards CD16$^+$ monocytes occurs only in end-stage...
renal disease, or at less severe renal impairment. Moreover, the prognostic impact of monocyte subset counts in non-dialysis CKD has been ignored before, despite the fact that non-dialysis CKD is an emerging global health issue, and that patients with less advanced CKD outnumber the dialysis population by far.

Albeit at lower CV risk compared with dialysis patients, individuals at earlier stages of CKD already suffer from an accelerated atherogenesis.23 Recently, the strong bidirectional relationship between renal and CV morbidity has been underscored by the introduction of the classification of cardiorenal syndrome, in which the pathophysiological role of monocytes has been especially highlighted.24

We now report that non-dialysis CKD patients have CD16+ monocyte counts close to the normal range observed in subjects with preserved renal function. Nonetheless, CD14++CD16+ monocyte counts are independently associated with CV events in patients with non-dialysis CKD in multivariate analysis.
In support of our present and earlier findings, there are other cogent arguments for the prominence of CD16+ monocytes but not CD16- monocytes in the inflammatory disease atherosclerosis: firstly, it is well established from epidemiological studies that CD16+ monocyte counts are elevated in many other inflammatory conditions. Secondly, CD16+ monocytes are efficient producers of inflammatory cytokines, whereas they poorly secrete the anti-inflammatory interleukin (IL)-10. Thirdly, several lines of evidence suggest a high endothelial affinity of CD16+ monocytes conferred by their surface expression of chemokine receptors and adhesion molecules e.g. CX3CR1, CCR5, VLA-4, and CD11c. Interestingly, it has already been reported in 2000 that CD16+ monocytes reside in the marginal pool where they can be rapidly mobilized in a catecholamine-dependent manner. This observation has been later verified in an outstanding study demonstrating that the mouse counterparts of CD16+ monocytes crawl along the endothelium and rapidly extravasate upon inflammatory stimuli. The authors termed this the ‘patrolling behaviour’ of the murine counterparts of CD16+ monocytes, and discussed these cells as a potential therapeutic target in inflammatory conditions such as atherosclerosis. In line with this notion, are mechanistic data from studies by Ancuta et al. indicating that CD16+ monocytes home to sites of endothelial activation in a CX3CR1-dependent manner, where they secrete MMP-9, CCL-2, and IL-6 with the ability to propagate further vascular injury through the recruitment of T-lymphocytes and additional monocytes. The relevance of CX3CL1 and CX3CR1 in atherosclerosis additionally makes a good case for the role of CD16+ monocytes in atherosclerosis, as CX3CR1 is highly expressed on CD16+ monocytes.

Further strong evidence derives from several studies showing that the CCR5 delta32 variant is associated with a more favourable CV outcome in the general population and with better all-cause as well as CV survival in patients with end-stage renal disease. CCR5 blockade in experimental atherosclerosis has been proved to be beneficial and has been consecutively discussed as a potential therapeutic option in diabetics as a CV high-risk population. Interestingly, CCR5 inhibition is feasible as it is an already applied therapeutic principle in HIV infection treatment. As pointed out before, CCR5 is expressed by CD16+ monocytes (especially CD14++CD16+ monocytes) but not by CD14++CD16+ monocytes as shown in the present study and previously.

The cumulative evidence for a prominent role of CX3CR1 and CCR5 in atherosclerosis—favouring CD16+ monocytes as drivers of atherosclerosis—together with our clinical data on the association between CD16+ monocytes, CV risk factors in low-risk subjects, and CV events in high-risk individuals and in the present trial, strongly suggest that CD16+ monocytes are most likely the relevant monocyte subset in human atherosclerosis. Furthermore, we feel that our data from non-dialysis CKD patients might be hypothesis generating for studies on monocyte heterogeneity and CV complications in the general population.

In summary, we report the role of CD14++CD16+ monocytes for future CV events in a cohort representative of a much larger patient group compared with earlier studies. Extending previous knowledge on monocyte heterogeneity and CV outcome, we raise now the hypothesis that modulating CD14++CD16+ monocyte function—e.g. through interference with the CCR5–CCL5 axis—might be beneficial for preventing CV events.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

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**Conflict of interest:** none declared.

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