Editorial comment: variables affecting the presence of mesenchymal stromal cells in the peripheral blood and their relationship with apheresis product

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In this issue of the British Journal of Haematology, Jain et al. identified circulating mesenchymal stromal cells (MSCs) in peripheral blood (PB) and apheresis product (AP) collected in the context of haematopoietic stem cell (HSC) mobilization for HSC transplantation (HSCT). As pointed out by the authors themselves and others (Fox et al., 2007; Jones & McGonagle, 2008; Moll et al., 2019), the presence of circulating MSCs in PB is debatable and their identification may be hampered, among others, by: (i) their low frequency in peripheral blood, (ii) their unclear phenotypic characterization, and (iii) the large biological variations related to donor pathology, disease status, and corresponding treatment regimens.

Within the light of these challenges, it is still not clear today if MSCs naturally extravasate and circulate within blood, or if the presence of MSCs in the circulation is a mere artefact resulting from the blood sampling procedure (e.g. vessel puncture), major micro- or macrovascular injury (e.g. severe fractures or surgery), or non-physiological mobilization strategies (e.g. mobilization drug toxicity) (Marquez-Curtis et al., 2011). Either point could likely lead to an artificial presence of perivascular MSCs in the blood stream, resulting from the breakdown of vascular barrier integrity (Alm et al., 2010; Hoogduijn et al., 2014; Moll et al., 2019).

Jain et al. investigated if MSCs can be mobilized into the blood stream in response to drug regimens already approved/applied for HSC collection in HSCT. Two reviews give an elaborate outline on the strategies used for progenitor cell mobilization (Marquez-Curtis et al., 2011; To et al., 2011). One prime example comprises the targeting of cytokine/chemokine and growth factor signalling with granulocyte colony-stimulating factor (G-CSF) and the CXCR4-antagonist plerixafor (AMD3100) for the modulation of the stromal-derived factor-1a (SDF-1a)/CXCR4-signalling axis that controls the retention of HSCs in the bone marrow (BM) (Fig 1).

Intriguingly, Larsen et al. noted in a baboon model that MSC mobilization and colony-forming unit fibroblast (CFU-F) in PB in response to G-CSF did only occur when adding stem cell factor (To et al., 2011). Rankin et al. found in mice, that MSCs/CFU-F were not found in PB post-mobilization with G-CSF, but when adding vascular endothelial growth factor and CXCR4-antagonist (Pitchford et al., 2009).

Nonetheless, first reports exist that documented small quantities of MSCs/CFU-F in PB of G-CSF-mobilized patients (Wexler et al., 2003; Kassis et al., 2006). This is in line with reports implicating the SDF-1a/CXCR4-axis in BM retention of various progenitor cell linages, but also in chemotraction of CXCR4-expressing BM progenitors to sites of tissue damage and ischaemia through hypoxia-inducible factor-1 (HIF-1)-mediated induction of SDF-1a (Petit et al., 2002; Ceradini et al., 2004; Kucia et al., 2005; Otsuru et al., 2008; Yin et al., 2010).

Jain et al. here report for the first time the detailed kinetics of: (i) circulating MSCs in PB of HSCT patients before HSC mobilization, (ii) in AP obtained at day 5 post start of mobilization with G-CSF (Grafeel, 10 µg/kg/day for 4 days), and (iii) in PB and AP at day 6 upon mobilization with G-CSF (10 µg/kg on day 6) and plerixafor (0.24 mg/kg given 12 hours prior), obtained in a second round of apheresis for those with inadequate CD34+ counts in the first apheresis (<2 × 10³/µl/kg).

To identify CD34+ HSCs and CD34dim MSCs, and to calculate their frequency in blood relative to white blood cells (WBCs), Jain et al. employed a dual platform strategy based on multiparameter flow cytometry and total leukocyte
counts. The MSCs were identified as CD34^dim/CD73^+/CD90^-/CD105^- cells, with dual positivity for either CD73^-/CD105^+ or CD73^-/CD90^-/CD105^- . The flow cytometry gating strategy and controls are shown in the primary article and the MSC frequency was calculated as follows: MSC (%) = (MSC Number/100)/Viable WBCs.

The flow cytometry was performed according to Hass et al. (2011) in line with the current guidelines for the use of flow cytometry and cell sorting. In addition, another method to enrich MSCs from blood is elutriation based on their physical properties (e.g. size/density) in a liquid centrifuge (Zvaifler et al., 2000). Furthermore, adhesion to fibrin micro-beads may also be used for MSC enrichment before further phenotyping with flow cytometry and other methods (Kassis et al., 2006).

Considering both patient background and sampling time points, the levels of MSCs detected by Jain et al. in PB and AP relative to viable WBCs were very similar in all three groups: healthy controls (0.0013—0.0033% MSCs in >50% of the n = 8 patients tested positive), multiple myeloma group (0.0011—0.0041% MSCs in >35% of the n = 20 patients), and lymphoma group (0.0016—0.0077% MSCs in >60% of the n = 5 patients). The typical MSC-like fibroblastic phenotype of the cells was confirmed upon plating of the sorted cells in vitro onto standard cell and tissue culture plastic.

Although the authors found small amounts of circulating MSCs in all three groups of donors, their relative number, expressed as percentages of MSCs relative to viable WBCs, did not appear to be increased in PB post G-CSF treatment. Here, the mathematical expression chosen by Jain et al. may carry a risk of misinterpretation, since WBC numbers in PB have been shown to be increased in response to G-CSF (De Felice et al., 2016; Melve et al., 2018). This may suggest that the absolute numbers of circulating MSCs may have actually increased in response to G-CSF.

Noteworthy, HSCs, endothelial progenitor cells (EPCs) and MSCs are not the only stem cell niches mobilized upon G-CSF treatment (Melve et al., 2018). Immune memory cells, like memory B cells, T central memory and T stem cell memory cells are also affected. Thus, a closer look at subsets of bone marrow-resident cells may yield great potential for more targeted mobilisation strategies as a new research directive.

In analogy to identification of MSCs within tissues (Mendez-Ferrer et al., 2010; Consentius et al., 2018), a crucial...
aspect for flow cytometry-based identification of circulating endothelial, haematopoietic, or mesenchymal progenitor lineages is their clear phenotypic distinction with appropriate marker panels (Pitchford et al., 2009). Of importance is their distinction from circulating HSCs (Mendez-Ferrer et al., 2008), circulating EPCs and also circulating mature endothelial cells (CEC) (Asahara et al., 1997; Lanuti et al., 2018; Farinacci, et al., 2019). In particular, the latter two are phenotypically closely related to MSCs, also adhere to culture plastic, and may therefore potentially easily be mistaken for MSCs.

A typical marker for identification of EPCs is the endothelial marker CD31, commonly employed to distinguish MSCs from endothelial cells (ECs) (Dominici et al., 2006), which was not included in the current panel of Jain et al. However, a major contamination with EPCs is unlikely, due to the exclusion of CD34+ cells (Farinacci et al., 2019). In line with the confirmation of the MSC identity as plastic-adherent fibroblastic cells, another concern is the potential phenotypic transformation of circulating progenitor cells once plated on tissue culture plastic, akin to the processes of endothelial or epithelial to mesenchymal transition (EndMT/EMT) (Krenning et al., 2010).

In conclusion, the studies of circulating MSCs in PB of humans are more than two decades old (Levesque et al., 2007). Most investigators agree that their frequency in blood is very low in healthy individuals, but that the amounts of circulating MSCs may increase under challenging conditions, thus supporting the notion that MSCs can be transiently found ‘circulating’ in blood. Jain et al. here provide further evidence that MSCs can be found in PB and AP of patients treated with a typical G-CSF-based MSC mobilization regimen. Further phenotyping of circulating MSCs and studies on the mechanism of MSC mobilization should be conducted.

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Conflicts of interest

All the authors declare that they have no conflicts of interests.

References

Alm, J.I., Koivu, H.M., Heino, T.J., Hentunen, T.A., Laitinen, S. & Aro, H.T. (2010) Circulating plastic adherent mesenchymal stem cells in aged hip fracture patients. Journal of Orthopaedic Research, 28, 1634–1642.

Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schattenberg, G. & Isner, J.M. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science, 275, 964–967.

Ceradini, D.J., Kulkarni, A.R., Callaghan, M.I., Tepper, O.M., Bastidas, N., Kleinan, M.E., Capla, J.M., Galiano, R.D., Levine, J.P. & Gurtner, G.C. (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nature Medicine, 10, 858–864.

Consentius, C., Mirenska, A., Jurisch, A., Reinke, S., Scharm, M., Zenchlussen, A.C., Hennig, C. & Volk, H.D. (2018) In situ detection of CD73+ CD90+ CD105+ lineage: mesenchymal stromal cells in human placenta and bone marrow specimens by chipcytometry. Cytometry Part A, 93, 889–893.

De Felice, L., Agostini, F., Suriano, C., Fraboni, D., Gregori, C., Tirindelli, M.C., Picardi, A., Santarone, S., Di Piazza, F., Di Bartolomeo, P. & Arcese, W. (2016) Hematopoietic, mesenchymal, and immune cells are more enhanced in bone marrow than in peripheral blood from granulocyte colony-stimulating factor primed healthy donors. Biology of Blood and Marrow Transplantation, 22, 1758–1764.

Dominici, M., Le Blanc, K., Mueller, L., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prokop, D. & Horwitz, E. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 8, 315–317.

Farinacci, M., Krahn, T., Dinh, W., Volk, H.D., Dungen, H.D., Wagner, J., Konen, T. & von Ahsen, O. (2019) Circulating endothelial cells as biomarker for cardiovascular diseases. Research and Practice in Thrombosis and Haemostasis, 3, 49–58.

Fox, J.M., Chamberlain, G., Ashton, B.A. & Middleton, J. (2007) Recent advances into the understanding of mesenchymal stem cell trafficking. British Journal of Haematology, 137, 491–502.

Hass, R., Kasper, C., Bohm, S. & Jacobs, R. (2011) Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. Cell Communication and Signaling, 9, 12.

Hoogdijin, M.J., Versteegen, M.M., Engela, A.U., Korevaar, S.S., Roemeling-van Rhijn, M., Merino, A., Franquela, M., de Jonge, J., Ijzermans, J.N., Weimar, W., Betjes, M.G., Baan, C.C. & van der Laan, L.J. (2014) No evidence for circulating mesenchymal stem cells in patients with organ injury. Stem Cells and Development, 23, 2328–2335.

Jones, E. & McGonagle, D. (2008) Human bone marrow mesenchymal stem cells in vivo. Rheumatology (Oxford), 47, 126–131.

Kassis, I., Zangi, L., Rivkin, R., Levinsky, L., Samuel, S., Marx, G. & Gorodetsky, R. (2006) Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. Bone Marrow Transplantation, 37, 967–976.

Krenning, G., Zeiberg, E.M. & Kalluri, R. (2010) The origin of fibroblasts and mechanism of cardiac fibrosis. Journal of Cellular Physiology, 225, 631–637.

Kucia, M., Reca, R., Miekus, K., Wanzek, J., Wojakowski, W., Janowska-Wieczorek, A., Ratajczak, J. & Ratajczak, M.Z. (2005) Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1–CXCR4 axis. Stem Cells, 23, 879–894.

Lanuti, P., Simeone, P., Rotta, G., Almici, C., Avvisati, G., Azzaro, R., Bologna, G., Budillon, A., Di Cerbo, M., Di Gennaro, E., Di Martino, M.L., DiDato, A., Doretto, P., Ercolino, E., Falda, A., Gregori, C., Leone, A., Losa, F., Malar, N., Marini, M., Maistroroberto, P., Mollace, V., Morelli, M., Muggiana, E., Musolin, G., Neva, A., Pierdomenico, L., Pinna, S., Piovani, G., Rocca, M.S., Russo, D., Scotti, L., Tirindelli, M.C., Trunzo, V., Venturella, R., Vitagliano, C., Zullo, F., Marchisio, M. & Miscia, S. (2018) A standardized flow cytometry network study for the assessment of circulating endothelial cell physiological ranges. Scientific Reports, 8, 5823.
Levesque, J.P., Winkler, I.G., Larsen, S.R. & Rasko, J.E. (2007) Mobilization of bone marrow-derived progenitors. *Handbook of Experimental Pharmacology*, 180, 3–36.
Marquez-Curtis, L.A., Turner, A.R., Sritharan, S., Ratajczak, M.Z. & Janowska-Wieczorek, A. (2011) The ins and outs of hematopoietic stem cells: studies to improve transplantation outcomes. *Stem Cell Reviews and Reports*, 7, 590–607.
Melve, G.K., Ersvær, E., Eide, G.E., Kristoffersen, E.K. & Bruserud, O. (2018) Peripheral blood stem cell mobilization in healthy donors by granulocyte colony-stimulating factor causes preferential mobilization of lymphocyte subsets. *Frontiers in Immunology*, 9, 845.
Mendez-Ferrer, S., Lucas, D., Battista, M. & Frenette, P.S. (2008) Haematopoietic stem cell release is regulated by circadian oscillations. *Nature*, 452, 442–447.
Mendez-Ferrer, S., Michurina, T.V., Ferraro, F., Mazloom, A.R., Macartthur, B.D., Lira, S.A., Scadden, D.T., Ma’ayan, A., Enikolopov, G.N. & Frenette, P.S. (2010) Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*, 466, 829–834.
Moll, G., Ankrum, J.A., Kamhieh-Milz, J., Bieback, K., Ringden, O., Volk, H.D., Geissler, S. & Reiner, P. (2019) Intravascular mesenchymal stromal/stem cell therapy product diversification: time for new clinical guidelines. *Trends in Molecular Medicine*, 25, 149–163.
Otsuru, S., Tanai, K., Yamazaki, T., Yoshikawa, H. & Kandeya, Y. (2008) Circulating bone marrow-derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway. *Stem Cells*, 26, 223–234.
Petit, L., Szerper-Kravitz, M., Nagler, A., Lahav, M., Peled, A., Habler, L., Ponomaryov, T., Taichman, R.S., Arenazana-Seisdedos, F., Fujii, N., Sandbank, J., Zipori, D. & Lapidot, T. (2002) G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nature Immunology*, 3, 687–694.
Pitchford, S.C., Furer, R.C., Jones, C.P., Wengner, A.M. & Rankin, S.M. (2009) Differential mobilization of subsets of progenitor cells from the bone marrow. *Cell Stem Cell*, 4, 62–72.
To, L.B., Levesque, J.P., Herbert, K.E., Winkler, J.G., Bendall, L.J., Hiwase, D.K., Antonenas, V., Rice, A.M., Gottlieb, D., Mills, A.K., Rasko, J.E., Larsen, S., Beligaswatte, A., Nilsson, S.K., Couney, J.P., Cambareri, A.C. & Lewis, I.D. (2011) Mobilisation strategies for normal and malignant cells. *Pathology*, 43, 547–565.
Wexler, S.A., Donaldson, C., Denning-Kendall, P., Rice, C., Bradley, B. & Hows, J.M. (2003) Adult bone marrow is a rich source of human mesenchymal ‘stem’ cells but umbilical cord and mobilized adult blood are not. *British Journal of Haematology*, 121, 368–374.
Yin, Y., Zhao, X., Fang, Y., Yu, S., Zhao, J., Song, M. & Huang, L. (2010) SDF-1alpha involved in mobilization and recruitment of endothelial progenitor cells after arterial injury in mice. *Cardiovascular Pathology*, 19, 218–227.
Zvaifler, N.I., Marinova-Mutafchieva, L., Adams, G., Edwards, C.J., Moos, J., Burger, J.A. & Mainini, R.N. (2008) Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Research*, 2, 477–488.