Circulating Plasma Cells in Monoclonal Gammopathies

Cirkulující plazmocyty u monoklonálních gamapatií

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Summary

Background: Monoclonal gammopathies are characterized by presence of clonal plasma cells in the bone marrow, although peripheral blood circulating plasma cells can be found in a significant proportion of patients. The number of circulating plasma cells is an independent prognostic marker associated with shorter survival, but it can also help to predict early relapse. The reason and mechanism of plasma cell expansion from the bone marrow to enter peripheral blood is still not entirely clear, but possible changes in the expression of adhesion molecules are probably involved. Multiparametric flow cytometry allows simple and exact enumeration of circulating plasma cells in different types of cell suspensions, even in their low quantity. The phenotype profile and confirmation of clonality regarding to their bone marrow clonal counterparts should be verified as well. There is no uniform method used in clinical laboratories for circulating plasma cells analyses at this moment. ***Aim:*** Review is focused on use of multiparametric flow cytometry for circulating plasma cells analysis in peripheral blood. It is comparing possibilities of their detection by different methods and on clinical relevance of that assessment. The standardization of analyses is the main goal. ***Conclusion:*** Multiparametric flow cytometry is a very sensitive method for detection of circulating plasma cells, so using a standardized approach can lead to determination and implementation of the flow cytometry diagnostic threshold in plasma cell leukemia suspicious cases as well as in prognostication of monoclonal gammopathies patients. Moreover, analysis of plasma cells phenotypic profile could probably clarify their future behaviour.

Key words

monoclonal gammopathies – circulating plasma cells – plasma cell leukemia – flow cytometry

Souhrn

Východiska: Monoklonální gamapatie jsou charakteristické přítomností klonálních plazmocytů v kostní dřeni, nicméně cirkulující plazmatické buňky lze u významné části pacientů nalézt i v periferní krvi. Počet cirkulujících plazmatických buněk je nezávislým prognostickým faktorem asociovaným s kratším přežíváním, ale také může napomoci předvídat časný relaps. Příčina a mechanizmus vycestování klonálních plazmocytů z kostní dřeně stále není objasněna, nicméně může zahrnovat např. změny v expresi adhezivních molekul. Multiparametrická průtoková cytometrie umožňuje jednoduché a přesné stanovení zastoupení cirkulujících plazmocytů v jakékoli buněčné suspenzi, a to i při velmi nízkých počtech, vč. stanovení jejich fenotypu a potvrzení příslušnosti ke klonálním plazmocytům kostní dřeně. V současnosti však v klinických laboratořích není používán jednotný postup k analýze cirkulujících plazmocytů. ***Cíl:*** Souhrnná práce popisuje využití průtokové cytometrie v analýze cirkulujících plazmocytů v periferní krvi. Zaměřuje se na možnosti detekce pomocí různých přístupů a také na klinický význam stanovení těchto buněk s cílem standardizace analýz. ***Závěr:*** Multiparametrická průtoková cytometrie je vhodnou a dostatečně citlivou metodou pro detekci cirkulujících myelomových klonálních plazmocytů. Využití standardizovaného přístupu může vést ke stanovení a zavedení nového „průtokově cytometrického" diagnostického kritéria u suspektních případů plazmocelulární leukemie, a může být využito také v rámci prognostikace pacientů s monoklonální gamapatií. Mimoto, stanovení fenotypového profilu klonálních plazmocytů by mohlo napomoci objasnit jejich budoucí chování.

Klíčová slova

monoklonální gamapatie – cirkulující plazmatické buňky – plazmocelulární leukemie – průtoková cytometrie

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Introduction

Presence of circulating "myelomatous" plasma cells (PCs) has an independent prognostic value in multiple myeloma (MM) [1,2]. Plasma cell leukemia (PCL) represents a unique subset of MM; it is generally a rare and aggressive PC proliferative disorder [3–5]. Existing methods of circulating plasma cell (cPC) detection provide correlating results in some cases but they are not comparable in manner of sensitivity and number of identified cPCs [6]. Although phenotypic profile of cPCs is at least partially known, information explaining behaviour of cPCs is needed. New sensitive multiparametric flow cytometry (MFC) based techniques are able to detect even low number of cPCs together with clonality assessment, so using a standardized approach may lead to improvement in diagnostics and clinical research [7].

Development and localization of PCs under normal and pathological conditions

PCs are terminal stage of B-lymphocytes differentiation with ability to produce antibodies. Their development takes place in secondary lymphoid organs, where B-cells are activated by the antigen and differentiate into plasmablasts. These progenitors of PCs migrate to the bone marrow (BM) where they interact with the BM microenvironment and differentiate into long-lived PCs [8–12]. Some studies described the presence of circulating antibody-secreting cells and especially their increasing number after immunization. Although part of the $CD19^{+}CD20^{-}CD38^{++}$ cells in blood might express CD138, a marker of PCs, they are probably not fully mature PCs but rather a transitional stage between plasmablasts and BM PCs [9–11]. These cells have homogenous $CD45^{bright}$ expression in all cases [12]. Typical transient expansions of polyclonal plasmablasts/early PCs can be observed in peripheral blood (PB) in case of reactive plasmacytosis in both neoplastic and non-neoplastic conditions [12,13]. Mature long-lived PCs in healthy donors persist only in the BM compartment, and they are not detectable in PB [14].

On the other hand, in monoclonal gammopathies (MG), characterized by presence of clonal PCs in BM, pathological PCs can expand into PB as cPCs [15,16]. Presence of cPCs was documented in various cases, not only in symptomatic MM, but also in asymptomatic smoldering MM (SMM), benign monoclonal gammopathy of undetermined significance (MGUS) [2,17,18] and also in patients with primary amyloidosis [19]. The cause and mechanism of PC expansion from the BM into circulation remains largely unknown, but the critical feature of cPCs is their independence from the BM microenvironment. Thus, some possible reasons of their migration may include changes in angiogenesis and microvessel density with subsequent increase in proliferative rate, higher incidence of cytogenetic abnormalities and changes in expression profile of adhesion molecules [14,20–23]. The question is if presence of cPCs is connected with natural development of the disease to the late phase of MM or forms a completely distinct biological group [21–23]. It was shown that cPCs are mostly quiescent, but surprisingly they have a higher clonogenic potential than their paired BM counterparts. This fact could explain their ability to disseminate into various locations in the BM. This suggests that cPCs represent a unique subpopulation coming from BM clonal PCs [22].

Detection of cPCs

Conventional morphology is able to detect cPCs in around 15% of all newly diagnosed MM patients [21,24]. Immunofluorescence microscopy (IM) detects cPCs in approximately 19–28.5% of MGUS patients, 25–38% SMM and more than 70% of newly diagnosed MM patients [1,15,25,26]. On the other hand, MFC can revealed presence of cPCs in 20–25% of MGUS patients, 40–69% of SMM patients and in 70–84% of newly diagnosed MM patients [2,14,25,27–29] and more than 90% of patients in relapse [14]. Using routine diagnostic immunophenotyping with sensitivity 10^{-4} was able to detect cPCs in 69% (78/113) newly diagnosed MM (median 0.2% and range 0.05–36.05) (own unpublished results). Using the more sensitive next generation flow (NGF) method, based on standardized Euroflow settings, presence of cPCs was documented in 60% of MGUS, 75% SMM and 96% of MM; and very recently cPCs were detected even in 100% SMM and MM new diagnosed patients [30,31]. Above that, using NGF and gene expression profiling (GEP) demonstrated that genetic features of cPCs are in concordance with BM clonal PCs [30]. It was recently published that CD138-based microfluidic PC capture is a potentially useful tool in MM as it permits quantitation of rare cPCs in blood and subsequent fluorescence-based assays [32]. Underestimation of PC number by MFC when compared to morphology evaluation is known, but results obtained by both methods correlate, and the percentage of PCs provided by MFC is also an independent prognostic factor affecting the overall survival (OS) of patients [6]. Hence, new "flow cytometric" criterion for PCL diagnostics should be established.

Characterization of cPCs by MFC

PCs are commonly identified using markers CD45, CD38 and specific CD138. Despite downregulation of CD138 on abnormal cPCs [22], they are clearly distinguishable by specific SSC/FSC position, usually bright expression of CD38 and low or absent expression of CD45 [14,33]. Discrimination of cPC from normal plasmablasts is important, especially in clonal $CD19^{+}$ MG cases (Fig. 1).

Aberrantly expressed or missing markers on clonal PCs have been described, as well as their relationship to prognosis of MG patients [34,35]. In comparison to BM PCs, cPCs show downregulation of some surface markers, such as CD11a, CD11c, CD29, CD49d, CD49e, CD33, CD56, CD117, CD138, CD28, CD38, CD81 and upregulation of CD44 and CD97, but their basic phenotype profile is mostly the same as in BM [14,22,36].

Adhesive molecule CD56, neural cell adhesion molecule (NCAM), seems to have a specific significance in abnormal PCs. CD56 is mainly involved during normal embryogenesis and has an important role in migration, mediates cell-to-cell interactions and cell-matrix interaction [37–39]. CD56 is commonly

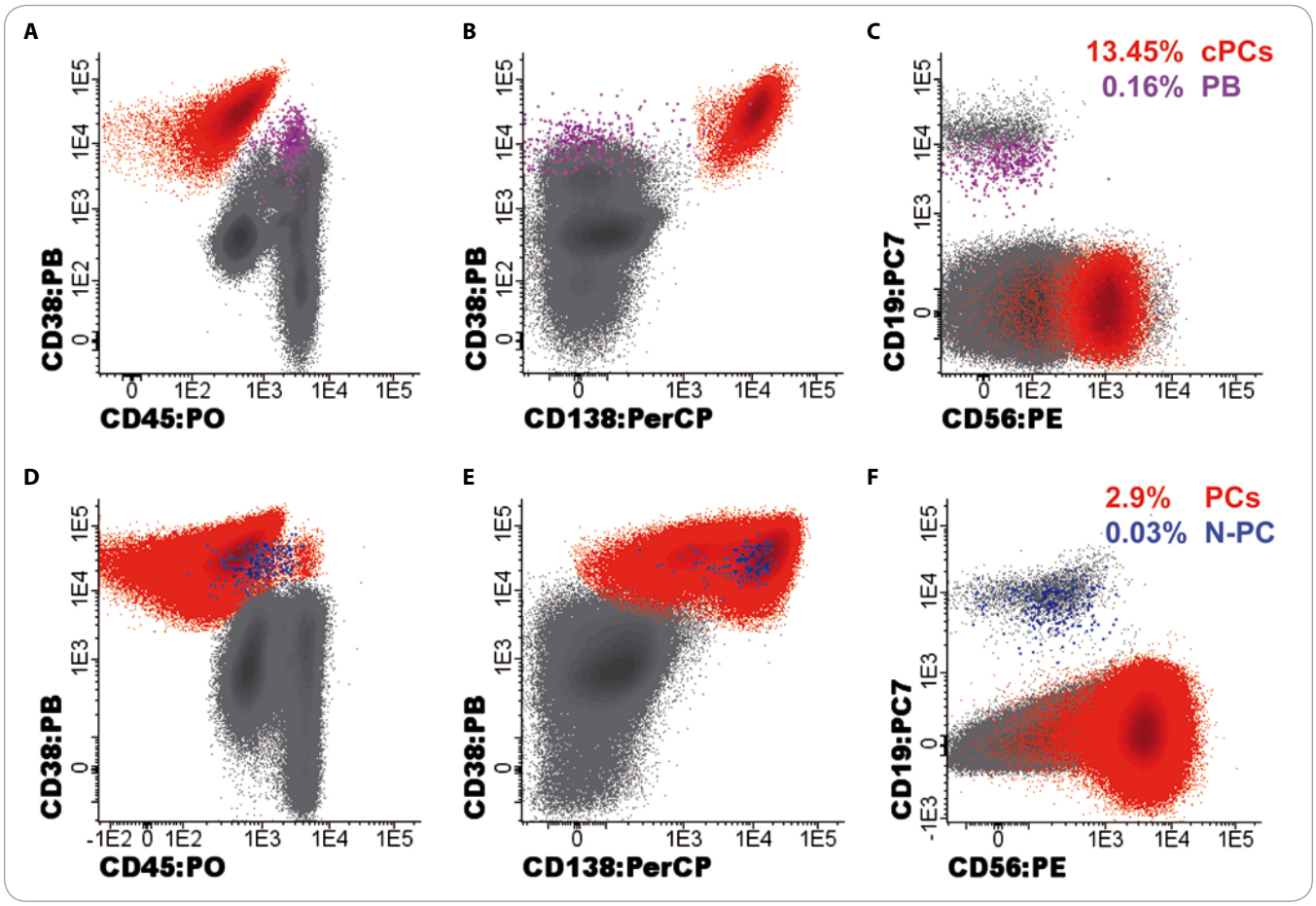

Fig. 1. Comparison of cPCs and PCs in a single patient.

The same phenotypic profile as $CD19^{-}CD56^{+}CD45^{-}$ was found when cPCs and PCs (red dots) were analyzed in whole PB (A–C) and BM (D–F), resp. Presence of plasmablasts is visible in PB (purple dots) and rezidual normal PCs (N-PCs) are present in BM (blue dots). Data acquired on BD FACS Canto II (BD Biosciences), reanalysis done in SW Infinicyt (Cytognos).

present on NK cells, but it is usually expressed on abnormal PCs in majority of MM [23,37,40]. There is no significant alteration of CD56 expression over the course of the disease, so it is possible that weak expression and/or absence of this molecule on PCs at the time of diagnosis may be connected to PCL development and can help predict dissemination of PCs into PB [23]. This is consistent with downregulation of CD56 in patients with extramedullary involvement [39,40]. Interestingly, CD56 negativity is related to the absence of lytic bone lesions [23,41] and is also associated with more aggressive disease and shorter OS in patients treated by conventional therapy [37,40]. However, lack of CD56 is not a prognostic marker in patients treated with high-dose chemotherapy, but is associated with t(11;14) [42]. Although decreasing and/or missing expression of CD56 was thought to be responsible for extramedullary spread, there are MG cases with clearly $CD56^{+}$ cPCs [39,40,43,44]. Also our results showed that CD56 was expressed in majority of cPC^{+} MM cases although median fluorescence intensity of CD56 in PB was lower than in BM. In our hands, the phenotypic profile of cPCs in a single patient is comparable to their clonal BM PCs (unpublished data).

Clinical relevance of cPCs

Presence of cPCs is associated with remarkably increased risk of malignant transformation of asymptomatic MGUS [1] and SMM into symptomatic MM [45]. Thus, MFC could be a convenient method to identify SMM patients with high-risk of progression before they develop end-organ damage [18]. It has also been shown that presence of cPCs in MGUS patients is related to shorter progression-free survival (PFS) and OS [1]. SMM patients with high levels of cPCs have significantly shorter OS compared to patients without cPCs or low number of cPCs [18,45]. It was demonstrated that MM patients with standard risk cytogenetics have better prognosis when associated with a lower number of cPCs, whereas those with high-risk cytogenetics had poor prognosis independently of number of cPCs [27]. Presence of cPCs positively correlates with higher level of beta2microglobulin, advanced ISS and Durie-Salmon stage and lower hemoglobin [21]. Moreover, their presence is also associated with shortened survival, so they could predict more aggressive disease and early relapse as well [20,46,47]. Thus, cPCs are a marker of

disease activity [48]. Number of cPC correlates with disease stage, and patients responding to therapy show reduction of cPCs [14]. Further, negative prognostic impact of cPCs in relapsed or refractory MM was observed [49]. Median time to progression (TTP) was the shortest in patients with no reduction of cPCs compared to patients with cPCs reduction or no detectable cPCs after one cycle of therapy (51 vs. 258 vs. 581 days); median OS was the shortest in the group of patients without reduction of cPCs in comparison to other groups (308 vs. 856 vs. 1,006 days) [49]. Therefore, monitoring of cPCs reduction after first cycle of therapy can be a useful tool for identification of patients resistant to treatment [49]. Negative prognostic impact of cPCs presence prior to transplant on post-transplant response has been observed. Achievement of stringent complete response (sCR) in the group with cPCs presence was 15 vs. 38% in patients with no detectable cPCs before autologous stem cell transplantation (ASCT) [50]. Patients without cPCs were associated with better PFS and OS in comparison to patients with detectable cPCs before ASCT (median PFS in the group with cPCs/without cPCs was 15.1 vs. 29.6 months and OS was 41.0 months vs. not reached). Thus, monitoring of cPCs presence before transplant by FC could predict survival in newly diagnosed MM [50]. Additionally, there were no PCs detected in patients who reached complete remission (CR) [14,51]. Although some studies found statistically significant positive correlation between higher presence of cPCs and the number of PCs in the BM [21,25,52], it was not confirmed by other research groups [2,36,45,53]. So, presence of cPC probably cannot be predicted on the basis of high BM infiltration when different methods of analyses are used (morphology vs. flow cytometry etc.).

Surprisingly, Paiva et al. demonstrated that numbers of clonal cPCs fluctuate in circadian rhythms [22]. In addition to that, patients with low or no cPCs were more likely to have bone lesions than those with high cPC numbers [25]. Monitoring of cPCs kinetics during treatment can help predict early relapse or refractory myeloma and reveal resistance to therapy [49].

Detection, analysis and separation of cPCs is a much less invasive way for their further characterization and useful PB "liquid biopsy" should be a part of routine examination of MG patients which can supply BM aspiration.

Plasma cell leukemia

PCL is a very rare and aggressive form of plasma cell dyscrasias characterized by presence of cPCs. The diagnostic criteria are based on a relative number of cPCs exceeding 20% of the total leukocytes and/or on the absolute number of cPCs exceeding 2×10^9/L in PB [3–5].

PCL can be divided in two different groups. Primary PCL (pPCL) originates *de novo* with no previous evidence of MM and represents approximately 60% of all PCL cases with median age at diagnosis 55 years [54,55]. On the other hand, secondary PCL (sPCL) is a leukemic transformation of pre-existing MM with median age at diagnosis 65 years and significantly shorter OS than pPCL (1.3 vs. 11.1 months) [55]. The clinical course of both PCLs is very aggressive with bad prognosis, significantly shorter OS than MM and adverse prognostic factors in comparison to MM [56,57]. Treatment of PCL is similar to MM, but despite aggressive new treatment strategies which have improved OS of pPCL patients, it is still worse than MM patients [56–58]. Interestingly, patients with pPCL achieve CR after transplantation more likely, but their OS remains worse than MM patients [59]. On the contrary, sPCL is usually not responsive to any treatment modality [60].

Analogous to MM, PCs of both PCL types express CD38, CD138 and typically have no CD19 [23,56,61]. Despite low incidence of PCLs, some studies demonstrated differences in their PC phenotypic profile. In addition to markedly reduced levels of CD27, which is associated with an aggressive clinical course of the disease [62], lack of or weak expression of CD56 seems to be a characteristic feature of pPCL in both compartments (BM and PB) [23,62]. Increased levels of soluble CD95 and beta2microglobulin have been observed in PCL [63]. Primary PCL shows higher expression of CD20 and lower CD9, CD56, HLA-DR and CD117 compared to BM PCs in MM patients [56,64] and lower expression of CD40 in comparison to MGUS [63]. Interestingly, expression of CD23, which is specific for abnormalities of chromosome 11, is associated with pPCL [65]. As well as in pPCL, typical absence of CD19 and negativity or low expression of CD56 in PB or BM was found in sPCL. $CD56^{-/weak}$ phenotype is stable from time of diagnosis to disease progression [23]. As mentioned above, majority of MM patients express CD56 [23,62], so the group of patients with $CD56^{-/weak}$ expression can possibly delineate a special subset of MM [23,42]. However, even in the era of new drugs, there is no clear connection between dissemination of PCs and loss of CD56, but $CD56^-$ group might preferentially be the source of sPCL [23]. In addition to that, PCL shows high expressions of CD54, CD49d, CD29, CD126; in contrast to MM; PCL has uniform expression of CD44. Moreover, overexpression of nestin – a protein marker for neural stem cells – was found in both PCLs when compared to MM [66]. Surprisingly, expression of molecules CD18, CD11a, CD11b was lower on BM PCs and higher on cPCs, so these differences may explain hematogenic dissemination characterizing PCLs [61]. In comparison to untreated MM, PCs from progressing MM patients and BM PCs or cPCs from sPCL patients expressed lower levels or were negative for CD106 and the activated form of CD29, resp., with a weaker or zero ability to adhere to human fibronectin [67]. Phenotypic profile of pPCL and sPCL is not very different, except for a decrease of CD117 in pPCL and loss of CD19 and CD20 expression in sPCL [66]. CD28 seems to be able to distinguish pPCL from sPCL. It was found that expression of CD28 can be found in PB, but not in all cases in BM, and these PCs are more proliferative. Furthermore, in contrast with other markers, CD28 can be upregulated during disease evolution and correlates with tumor progression [23,64].

Using conventional morphology, a group of patients with the presence of

5–20% cPCs was observed to have worse OS independently of other prognostic factors, such as age, creatinine and the grade of D-S and ISS staging system [24]. Above that, this group of patients had lower platelets number together with higher BM infiltration by PCs. Consistently, another study found that OS of MM patients with more than 2% of cPCs was comparable to pPCL [21,24]. Based on this observation, it seems that the time has come to reconsider the diagnostic criteria of PCL. Also, due to the well-known phenomenon of underestimation of PC number by MFC when compared to morphology evaluation [6], and together with important role of MFC in PCL diagnostics [68], there is a need to determine the new flow cytometry criterion for PCL diagnosis which reduces previously established morphologic cut-off to (at least) 10%.

Conclusion

Presence and clinical significance of cPCs is still a partially unsolved phenomenon in majority of MGs. MFC is a very sensitive method not only for detection of cPCs, but also for determination of their phenotypic profile. Technical development of MFC together with development of novel standardized NGF analysis allows standardization of this approach. Further analysis of cPC using other methods on cellular and molecular level may further explore their characteristics regarding their possible future behaviour. Moreover, definition of the "flow cytometry criterion" for PCL diagnostics as well as the prognostication of patients according to cPCs presence and phenotype should be validated in the future.

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