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Fluidity and cytosolic Ca^{2+} concentration of circulating polymorphonuclear leukocytes at baseline in some chronic and acute clinical conditions: review of our survey

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Key words
PMN membrane fluidity – PMN cytosolic calcium concentration – hypertension – diabetes – chronic kidney disease – stroke – myocardial infarction

Abstract. Objective: In this mini-review we describe the behavior of polymorphonuclear leukocyte (PMN) membrane fluidity and of PMN cytosolic Ca^{2+} concentration in some chronic and acute clinical conditions. Methods: PMN membrane fluidity was evaluated employing the fluorescent probe Fura-2AM, and PMN cytosolic Ca^{2+} concentration was evaluated using the fluorescent probe TMA-DPH. Results: From the determination of these two parameters investigated on resting PMNs, an almost constant increase in PMN cytosolic Ca^{2+} concentration in chronic clinical conditions, such as vascular atherosclerotic disease with and without diabetes mellitus, essential hypertension, chronic kidney disease, and diabetes mellitus of both types, and a decrease in PMN membrane fluidity in acute clinical conditions, such as juvenile acute myocardial infarction and acute ischemic stroke, are evident. Conclusion: The possible reasons for this different behavior are analyzed on the basis of pathophysiological considerations.

Introduction

In the past decades many papers of our group have investigated the functional aspect of polymorphonuclear leukocytes (PMNs) in the following clinical conditions: vascular atherosclerotic disease, essential hypertension, chronic kidney disease (CKD), diabetes mellitus (DM), acute ischemic stroke (AIS), and juvenile acute myocardial infarction (AMI).

PMNs attract microrheological and metabolic attention because these cells, with their geometric and biological characteristics, significantly influence the microvascular flow and this effect is due in particular to their adhesion to the endothelial cells, to their entrapment, or to their spontaneous activation. At the same time many papers have instead focused on the role played by the leukocyte count in the above-mentioned clinical conditions [1, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 11, 12, 13, 14].

Regarding PMNs, our attention has been especially directed towards the examination of the PMN membrane fluidity and the PMN cytosolic Ca^{2+} concentration, both under basal condition and after in vitro activation with chemotactic agents, such as 4-phorbol, 12-myristate, 13-acetate (PMA), and N-formyl-methionyl-leucyl-phenylalanine (fMLP).

PMN membrane fluidity, related in particular to the membrane lipid and protein composition, is a major component of the PMN deformability, influenced also by cytosolic Ca^{2+} concentration. These two parameters affect the functional aspects of PMNs, considering that membrane fluidity regulates some functions of PMNs, such as phagocytosis, and that the increase in cytosolic Ca^{2+} concentration is considered a marker of PMN activation. It must be underlined that the cytosolic calcium concentration is related to the activity of the membrane pumps and that this activity is influenced by the membrane fluidity [15, 16].

According to our experience, at baseline these two PMN parameters show a different behavior in chronic and acute clinical conditions (Table 1).

Chronic clinical conditions

In chronic vascular atherosclerotic disease (VAD) with and without type 2 diabetes mellitus (DM2), we noted [17, 18, 19] an in-

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Table 1. Behavior of the PMN membrane fluidity and of cytosolic Ca^{2+} concentration in chronic and acute clinical conditions (previously published personal data).

Chronic conditions	PMN membrane fluidity	PMN cytosolic Ca^{2+} concentration
VAD with DM	↔ [17, 18, 19]	↑ [17, 18, 19]
VAD without DM	↔ [17, 18, 19]	↑ [17, 18, 19]
Hypertension	↔ [20, 21, 22]	↑ [20, 21, 22]
Chronic kidney disease	↔ [23, 24, 25, 26]	↑ [23, 24, 25, 26]
Diabetes mellitus	↔ [27, 28, 29, 30]	↑ [27, 28, 29, 30]
Acute conditions		
Acute ischemic stroke	↓ [35, 36, 37]	↔ [35, 36, 37]
Young myocardial infarction	↓ [38]	↑ [38]

VAD = vascular atherosclerotic disease; DM = diabetes mellitus.

crease in PMN cytosolic Ca^{2+} concentration without any significant variation of the PMN membrane fluidity. This trend was confirmed when the VAD subjects, with and without DM2, were also subdivided according to the monovascular or polyvascular localization of the disease. In addition, it has to be emphasized, while in normal controls and in VAD subjects without DM we found a significant positive correlation between PMN membrane fluidity and PMN cytosolic Ca^{2+} concentration, this finding was not observed in the group of VAD subjects with type 2 DM [18]. The same results have been noted in hypertensive [20, 21, 22] and in CKD patients [23, 24, 25, 26]. At baseline, in subjects with essential hypertension we found an increase in PMN cytosolic calcium content only. In our survey, in essential hypertension none of these two PMN parameters was correlated with systolic and diastolic blood pressure values; also in conservatively treated CKD patients, none of these PMN parameters was correlated with blood urea nitrogen or serum creatinine.

In diabetic disease, the behavior of the PMN membrane fluidity and cytosolic Ca^{2+} concentration seems to be controversial [27, 28, 29, 30], although the examination of these parameters suggests that they are especially dependent on the extent of the record of cases and on the glicometabolic pattern. Regarding this last aspect, we know in fact that the PMN dysfunction results significantly correlated to the PMN metabolic profile that characterizes diabetes mellitus. This profile regards in particular the decreased activity of phosphofructokinase, and thus the glycolytic pathway, the increase in the hexose monophosphate shunt, the activation of the polyol pathway, and thus the increased PMN content of sorbitol. Recently [31], re-examining a large group of diabetics of both types, we found that PMN membrane fluidity does not discriminate diabetics of both types from normal controls while PMN cytosolic Ca^{2+} content is especially significantly increased in patients with type 1 diabetes (Table 1).

Acute clinical conditions (ischemic stroke and juvenile myocardial infarction)

PMN membrane fluidity and PMN cytosolic Ca^{2+} concentration seem to show a particular trend in acute clinical conditions, such as acute ischemic stroke and juvenile acute myocardial infarction (Table 1).

We know that AIS is associated with brain infiltration of several types of inflammatory cells, including leukocytes [32], even if a direct action of leukocyte activity and inflammation in the ischemic lesion has not been demonstrated [14]. In AMI, the PMNs, besides being responsible for the inflammatory processes, are involved in the decreased microcirculation flow leading to negative events and in particular to the no-reflow phenomenon [33]. It has been demonstrated that PMNs also play a role in left ventricular remodeling after AMI [34].

In a first group of AIS subjects [35, 36, 37], examined 48 – 72 hours after the onset of stroke, we noted that only PMN membrane fluidity was significantly decreased in comparison with controls without any variation of the PMN cytosolic calcium concentration. In a second group (unpublished data) we noted that the decrease of the PMN membrane fluidity, besides being evident at the initial stage, was also present 30 days after the ischemic event; in these subjects we observed, at the initial stage and after 30 days, an increase in the leukocyte count and in particular in the PMN count.

In subjects with juvenile AMI we observed a decrease of PMN membrane fluidity associated with a significant increase in cytosolic Ca^{2+} concentration; this finding persisted 12 months later [38]. At the initial stage of juvenile AMI, the leukocyte count

was increased in comparison with controls and this increase was also evident after 12 months; the same trend was also noted for the PMN count [38].

Conclusive and pharmacological consideration

While the increase of PMN cytosolic Ca^{2+} concentration may be explained by a functional abnormality of PMN Ca^{2+} ATPase activity, the decrease of PMN membrane fluidity is probably dependent on the alteration of the lipid and protein composition of the PMN membrane that modifies the polarization degree of the fluorescent probe. In our research we employed the fluorescent probe TMA-DPH (1.4-(trimethylamino)-phenyl-4-phenylhexatriene), a cationic derivative of DPH, that is localized at the lipid/water interface region of the bilayer where it remains for up to 30 minutes. The data obtained using this fluorescent probe reflects in particular the membrane lipid fluidity that, partly influenced by the cholesterol/phospholipid ratio, regulates the PMN deformability.

We suppose that the decrease in PMN membrane fluidity observed in juvenile AMI and AIS might be ascribed to the alteration of oxidative status, in particular to lipid peroxidation and protein oxidation accompanying these events; the abnormal oxidative status contributes to the modification of the lipid ordering, especially in the outside part of the PMN membrane. In subjects with juvenile AMI in whom we investigated plasma lipid peroxidation, expressed as TBARS, and plasma protein oxidation, expressed as carbonyl groups, a marked increase in both parameters was found at the initial stage of AMI [39, 40]. The same behavior was present in AIS patients, in whom the increase in lipid peroxidation and protein oxidation, besides being evident at admission and 24 hours later [41], persisted for some months after the onset of AIS [42].

Also considering that PMN membrane fluidity and cytosolic Ca^{2+} concentration seem to have a particular behavior when activation techniques are employed in vitro, it is useful to underline their trend as an indicator of PMN dysfunction.

All these findings might suggest to choose the pharmacological treatment for each of these clinical conditions according to the molecules that are able to modulate or modify the PMN functional behavior, such as calcium channel blockers [43,44], pentoxifylline [45], prostacyclin analogs and mimetics [46], buflomedil [47], beta-blockers [48], and statins [49,50].

Furthermore, it must be underlined that in AMI subjects, monoclonal antibodies against β2-integrins (CD18 or CD11/CD18) were used to influence the functional activity of the PMNs [51, 52, 53, 54]. In AIS subjects an approach with monoclonal antibodies versus CD11/CD18 and CD11b/CD18 was employed instead [55, 56].

In conclusion, there is some information regarding the role of polymorphonuclear leukocytes in several clinical conditions, and at the same time there is much data regarding the influence of some molecules on some characteristics of these circulating cells; in this brief report based on our experience in the microrheological field, we have presented the behavior of these two PMN parameters in some chronic and acute diseases.

Conflict of interest

None.

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