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# Aggregability and disaggregability of erythrocytes in women suffering from ovarian cancer: evidence for an increased disaggregation threshold

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**Abstract.** In order to further characterize the alterations of erythrocyte aggregation described in ovarian cancer, we measured it with laser backscattering in eleven women suffering from ovarian cancer (mean age: 44.7 ± 3.6, extreme values: 28–61 yr) compared with thirteen matched control women. Blood rheology exhibited a wide variability in cancer patients, with some unusually high values of plasma viscosity and/or RBC aggregation in individual cases. The only significant differences were found for the RBC disaggregation threshold which was higher in patients than in controls (78.06 ± 10.14 vs 52.6 ± 3.15 $s^{-1}$, $p < 0.05$), while hematocrit was lower (34.45 ± 1.42 vs 38.23 ± 0.75, $p < 0.05$). A negative correlation between hematocrit and corrected blood viscosity on the whole sample of subjects ($r = 0.454$, $p < 0.05$) indicates that hematocrit is decreased in subjects prone to high viscosity, resulting in similar values of apparent blood viscosity in controls and patients. Thus, a lower disaggregability of RBCs is evidenced in women with ovarian cancer, as well as a tendency to blood hyperviscosity compensated by a reduction of hematocrit which suggests that there may be some degree of '*viscoregulation*'.

Keywords: Ovarian cancer, erythrocyte deformability, blood rheology, erythrocyte aggregation

## 1. Introduction

There are some reports indicating that blood rheology is impaired in patients with cancers, probably as a consequence of inflammatory processes [1]. Development of studies on blood rheology in cancer patients may have two interests: first, hemorheological disturbances are proportional to the tumor size and tumor type [2] and in some cases like melanoma have been suggested to be related to the evolutivity of the disease [3]. Thus they could be hypothesized to provide markers of evolutivity, beside the classical erythrocyte sedimentation rate which depends mostly on blood rheology but is also influenced by less defined parameters [1]. On the other hand, modifications of blood rheology may have a pathophysiological role which remains to be elucidated: are they involved in dissemination of metastatic cells after stasis? Are they, on the contrary, a reflect of a protective mechanism by inflammatory processes? May they increase the risk of deep venous thrombosis? [4].

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One of the most dangerous human cancers is the cancer of the ovary. In this cancer abnormalities in the plasma protein pattern have shown to be associated with alterations of blood rheology, including an increase in erythrocyte aggregability and a higher plasma viscosity. Therefore, we aimed at better analyzing these alterations in the aggregation properties of red cells with the laser backscattering technique, which allows the quantification of disaggregability and thus gives an idea of the strength of interactions among hyperaggregating red cells.

## 2. Materials and methods

### 2.1. Patients

We studied 11 women with ovarian cancer before the onset of treatment, compared with 13 matched women coming to the department of gynecology and obstetrics for the surgical treatment of benign gynecological lesions: uterine fibromyoma ($n = 8$); ovarian benign cyst ($n = 4$); vaginal cyst ($n = 1$). Ovarian cancers at the time of discovery were classified according to the classification of the International Federation of Gynecology and Obstetrics (FIGO) from stage I to stage IV and there was an equal number of cancers in the various stages (Table 1).

### 2.2. Laboratory measurements

Blood samples for hemorheological measurements (7 ml) were obtained with a large bore needle (Luer adaptor Venoject, set into the catheter) to avoid shear damage to erythrocytes. A vacuum tube was used for blood withdrawal, with potassium EDTA as the anticoagulant. No tourniquet was used for sample drawing in order to minimize venous stasis. Viscometric measurements were performed at high shear rate (1000 $s^{-1}$) with a falling ball viscometer (MT 90 Medicatest, 37 rue de l'Ermitage, F-86280 Saint Benoit) [5,6]. The coefficient of variation of this method ranges between 0.6 and 0.8% (10 repetitive measurements of the same sample). We measured with this device apparent viscosity of whole blood at native hematocrit and plasma viscosity. From these measurements we also calculated blood viscosity at corrected hematocrit (45%) according to the well-known equation of Quemada [7]:

$$\eta_b = \eta_{pl}(1 - (1/2)kh)^{-2},$$

where $\eta_b$ is blood viscosity, $\eta_{pl}$ plasma viscosity, $h$ the hematocrit and $k$ a shear dependent intrinsic viscosity of the red cells according to Quemada.

Two indices of erythrocyte rigidity (Dintenfass' 'Tk' and Quemada's 'k') were calculated from blood viscosity, hematocrit and plasma viscosity measured at time 0 with equations derived from those given above [1,7,8]:

$$k = 2(1 - \eta_r^{-0.5})h^{-1}$$

Table 1
Clinical characteristics of the 24 women of the study

| | Cancer ($n = 11$) | Controls ($n = 13$) | Comparison |
|---|---|---|---|
| Age (yr) | 45.7 ± 4.2 | 45.4 ± 4.6 | ns |
| Weight (kg) | 54.9 ± 1.5 | 55.7 ± 1.5 | ns |
| Height (m) | 1.62 ± 0.01 | 1.6 ± 0.015 | ns |
| Body mass index (kg/m$^2$) | 20.8 ± 0.4 | 21.7 ± 0.45 | ns |

and [8]

$$\mathrm{Tk} = (\eta_r^{0.4} - 1)(\eta_r^{0.4} h)^{-1},$$

where $\eta_r$ is relative blood viscosity $\eta_b/\eta_{pl}$.

The hematocrit/viscosity ratio, an index of oxygen supply to tissues, was calculated according to Chien [9] and Stoltz [10], with $h$ (as percentage) divided by $\eta_b$ value at high shear rate which was determined as described above. RBC aggregation was measured with the AFFIBIO aggregometer which is based upon the experiments of Mills [11–13] on cell disaggregation behavior in shear flow. This device measures the changes in backscattered light which are observed when sheared RBC suspensions are abruptly brought to a full stop. The decrease in the optical signal reflects the formation of RBC aggregates [12]. Some parameters are derived from the curve of light intensity as a function of time. The aggregation time is the reciprocal of the initial slope (calculated between 0.5 and 2 s after the shear has stopped). The aggregation index at 10 s is a measurement of the extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 10 seconds. This device also measures disaggregation thresholds, by submitting blood to a succession of shear rates from 600 $s^{-1}$ to 7 $s^{-1}$. The total disaggregation threshold is the shear rate below which the backscattered light intensity starts to decrease, indicating that the shear stress applied to aggregates is no longer sufficient for allowing complete dispersion of RBC aggregates. The partial disaggregation shear rate is defined as the shear rate corresponding to the intersection point of the two asymptotes drawn from the extremes (maximum and minimum shear rate). The Myrenne aggregometer [14] was also used, giving two indices 'M' and 'M1' of aggregation.

### 2.3. *Statistics*

Results are presented as mean ± the SE of the mean. A value of $p < 0.05$ was considered as significant. Correlations were performed using the method of least squares. Variables in the two groups were compared using the two tailed nonparametric test of Mann–Whitney for unpaired data.

## 3. Results

Comparisons between patients and matched control women are shown in Table 2. Although there was a wide variability, with some unusually high values of plasma viscosity and/or laser backscattering parameters of RBC aggregation in several patients, the only differences found were on RBC disaggregation threshold which was higher in patients than controls ($p < 0.05$), while hematocrit was lower ($p < 0.05$). As shown in Fig. 1 there was a negative correlation between hematocrit and corrected blood viscosity on the whole sample of subjects ($r = 0.454$, $p < 0.05$). This correlation means that hematocrit is decreased in subjects with high factors of viscosity, resulting in similar values of apparent blood viscosity in controls and patients.

## 4. Discussion

This study, which aimed at further analyzing the 'hemorheological profile' of patients with ovarian cancer, evidences a lower disaggregability of RBCs and a tendency to blood hyperviscosity compensated by a reduction of hematocrit.

Table 2
Hemorheological parameters measured in the 24 women of the study (mean ± SEM)

| | Cancer ($n = 11$) | Controls ($n = 13$) | Comparison |
|---|---|---|---|
| Whole blood viscosity (mPa.s) | 2.91 ± 0.12 | 3.07 ± 0.13 | ns |
| RBC aggregation "M" | 6.75 ± 0.71 | 6.01 ± 0.9 | ns |
| RBC aggregation "M1" | 9.69 ± 1.13 | 8.11 ± 1.16 | ns |
| Aggregation time (s) | 1.63 ± 0.25 | 1.79 ± 0.18 | ns |
| RBC disaggregation threshold ($s^{-1}$) | 78.06 ± 10.14 | 52.6 ± 3.15 | $p < 0.05$ |
| Hematocrit | 34.45 ± 1.42 | 38.23 ± 0.75 | $p < 0.05$ |
| Plasma viscosity (mPa.s) | 1.58 ± 0.07 | 1.66 ± 0.06 | ns |
| RBC rigidity "Tk" index | 0.633 ± 0.03 | 0.61 ± 0.03 | ns |
| Corrected blood viscosity Hct 45% (mPa.s) | 3.74 ± 0.18 | 3.69 ± 0.07 | ns |

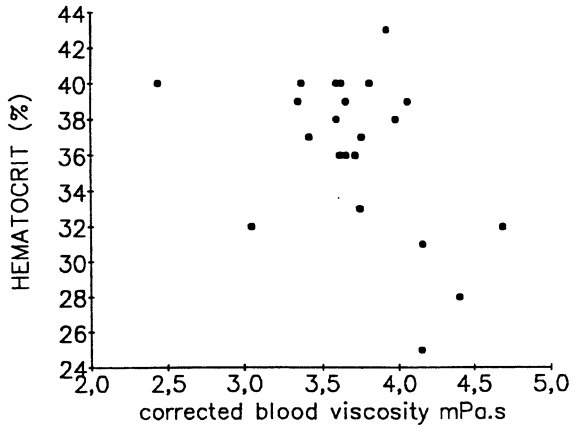


Fig. 1. Negative correlation between hematocrit and corrected blood viscosity on the whole sample of subjects ($r = 0.454$, $p < 0.05$).

The hemorheologic pattern of ovarian cancer has already been described by von Tempelhoff [4,15] who clearly evidenced a higher aggregability of red cells in this disease [15]. This finding is consistent with a series of 50 cancers of head and neck (compared to 80 controls) recently reported by Khan et al. [17] who evidenced a rise in plasma viscosity, red cell aggregation, red cell rigidity and plasma fibrinogen. Another report by Ranade and coworkers [18] also evidenced abnormalities of blood rheology in head and neck cancers and found that plasma viscosity became frankly non-Newtonian in critical patients, perhaps related to alterations of plasma components by substances released by the cancer. Another study by Karabanov and coworkers [19] examined blood rheology in 163 patients with gastric cancer and found abnormally high blood and plasma viscosity together with red blood cells hyperaggregation. Here again these changes were associated with an abnormal protein pattern.

Our study shows that the hyperaggregation already described by previous investigators in ovarian cancer [4,15] is associated with a lower ability to dissociate erythrocyte aggregates. We think that this finding is potentially important from a pathophysiologic point of view, since a reduced dissociation of aggregates at the entrance of the microvascular bed is likely to disturb blood distribution [1,9].

Actually, the exact biochemical mechanism of RBC hyperaggregation in cancer patients is not clear. In the paper of Khan et al. [17], end-stage tumors were associated with a rise in whole blood viscosity which was assumed to be due to a specific (unknown) factor released by the tumor. This hypothesis of a specific tumor factor is in agreement with the findings of von Tempelhoff [15] who observed higher RBC aggregability in ovarian cancer compared to breast cancer. However, this investigator [15] observes that, in ovarian cancers, the highest plasma viscosity values are mostly due to increased plasma protein content (D-dimer and fibrinogen). Another hypothesis can be made if we consider that this hemorheologic pattern of an increased disaggregation threshold, associated to almost unchanged other aggregation parameters, as found here, has been described after experimental exposure of RBCs to oxygen free radicals [16]. Therefore, free radical generation that may occur, during inflammatory processes, as a consequence of leukocyte activation, could provide an alternative explanation for our finding. Other studies including markers of oxidant stress will be needed to clarify this point.

Regardless the mechanism which remains to be clarified, rheologic abnormalities in cancer appear to be related to the evolution and prognosis of the disease. This does not appear clearly in our study, perhaps because of the relatively low number of patients, but there are several reports on larger series which strongly suggest that blood rheology may be a fair marker of evolutivity in several cancers [17,19], including the ovarian cancer [15]. Interestingly, treatment by chemotherapy improved this hemorheological pattern except when there were intercurrent thromboembolic events [20].

Rheologic alterations have also been reported to be predictive of deep venous thrombosis (DVT) in ovarian cancer [4,20]. The value of plasma viscosity before operation appears to be predictive of further DVT, while the postoperatory value has no predictive value [9]. It appears that, in ovarian cancer, a value of plasma viscosity below 1.34 mPa.s almost excludes the occurrence of a latter DVT [4]. In lung cancer, an algorithm including rheological parameters has been proposed by Skorniakov [21] after he studied 286 men subjected to pneumonectomy. This algorithm for predicting thrombotic complications based on the minimal set of informative criteria appeared to be accurate in 75% of patients before surgery and 82.2% of patients on day 1 postoperation [21]. This important aspect has not been investigated in our study.

Another interesting finding of our study is the correlation between hematocrit and corrected blood viscosity. Although this correlation is weakly significant ($p < 0.05$) it indicates that hematocrit is decreased in subjects with high factors of viscosity, so that apparent blood viscosity remains almost the same in controls and patients. A reduction in hematocrit has already been observed in ovarian cancer, in patients with high plasma viscosity who were prone to develop a further DVT [20]. This phenomenon of low hematocrit compensating a tendency to high viscosity has been observed in many clinical situations such as diabetes [22,23] and preeclampsia [24]. The late Leopold Dintenfass used to describe it as an homeostatic phenomenon of '*viscoregulation*' due to the regulatory effect of putative 'viscoreceptors' sensitive to hematocrit and viscosity [1]. More recently, some physiological support for this assumption has been given by the team of Reinhart [25,26] who reported that plasma viscosity inhibits erythropoietin response to anemia. Presumably, this mechanism which is assumed by these authors to explain the anemia developing in patients with increased plasma viscosity due to hypergammaglobulinemia, may be involved in the hematocrit-lowering effect of other situations of hyperviscosity, such as the cancers studied here.

On the whole, this study shows that the previously described hyperaggregation of red cells in women suffering from ovarian cancer is associated with a lower ability to dissociate aggregates, which is likely to have pathophysiological consequences. Moreover, the increase in blood viscosity factors described in

previous studies appears to be compensated by a reduction in hematocrit in most subjects, preventing to some degree the occurrence of an overt hyperviscosity syndrome.

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