Erythrocyte aging in the demented elderly: a fluctuating process?

G.J.C.G.M. Bosman a,*, P.A.J. Van der Linden b, I.G.P. Bartholomeus a, A.J.M. De Man c, W.J. De Grip a, P.J.C. Van Kalmthout b

a Department of Biochemistry, Institute of Cellular Signalling, Faculty of Medicine, University of Nijmegen, P.O. Box 9101, NL-6500 HB, Nijmegen, The Netherlands
b Nursing Home 'St. Joachim en Anna', Nijmegen, The Netherlands
c Blood Transfusion Service, University Hospital Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands

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Abstract

Measurement of erythrocyte aging parameters in patients with dementia indicates that an Alzheimer-related disturbance of the erythrocyte aging process may not be detectable until in the more advanced stages of the disease. Also, a strong fluctuation in the values of erythrocyte aging parameters, over a period of 15 months, was observed in patients with dementia, but not in age-matched control donors. This fluctuation was independent of the type and stage of dementia, and its cause remains to be elucidated. Such variability hampers the use of erythrocyte aging characteristics for the diagnosis of dementia. On the other hand, the aging-related erythrocyte IgG content may be a sensitive biomarker for disturbed systemic homeostasis in the elderly. © 1998 Elsevier Science Ireland Ltd.

Keywords: Aging; Alzheimer; Anion exchanger; Biomarker; Dementia; Erythrocyte; Immunoglobulin G

* Corresponding author. Tel.: +31 24 3615390/3614229; fax: +31 24 3540525; e-mail: G.Bosman@bioch.kun.nl

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1. Introduction

Recent findings support the theory that processes leading to disturbed cellular aging and untimely cell death are part of the etiology of Alzheimer’s disease (AD; Bosman and De Grip, 1996). In AD, a disturbance of the normal cellular aging process may not be restricted to the brain (Zatta and Nicolini, 1995). Structural and functional changes in the anion exchanger (band 3 or AE1) are central in the aging process of the human erythrocyte (Kay, 1993), and indications have been found for a disturbance of erythrocyte aging in patients in advanced stages of AD (Bosman et al., 1991). Subsequent studies in individuals with Down’s syndrome indicated that some erythrocyte aging parameters become abnormal in a presymptomatic stage (Bosman et al., 1993). In order to substantiate this conclusion, the following questions were addressed: (1) Do the erythrocyte band 3-related aging characteristics constitute a stable trait in healthy humans, or do they vary with age? (2) Are erythrocyte aging parameters affected by the stage of the disease in AD patients?

In view of the putative importance of this issue for early diagnosis, we repeatedly measured erythrocyte aging characteristics in patients with various types and in various stages of dementia in a period of 15 months after their admittance to a psychogeriatric nursing home. All patients (N = 35; 81 ± 5 years old) had a general diagnosis of dementia, with a Global Deterioration Scale (GDS) of 5 or more (Reisberg et al., 1982). Using the diagnostic criteria described previously (Bosman et al., 1991), patients were divided into four groups: senile dementia of the Alzheimer Type (SDAT), dementia of vascular origin (VASC), mixed type dementia (MIX), and atypical dementia (ATY). Mixed type dementia was defined when a vascular origin was suspected (e.g. hypodensity of vascular origin on CT scan, or a hypoperfusive incident), but with insidious onset, and atypical dementia was diagnosed when a clear choice between SDAT, VASC, and MIX could not be made (Koopmans, 1994). All groups were matched with respect to age, GDS, duration and stage of the disease, and use of medicine. During the course of this study, dementia progressed to a similar extent in the various groups, as indicated by an increase in the mean GDS scores of one to two. Control groups consisted of age-matched (AMC; N = 10; 85 ± 5 years old) and young (YC; N = 8; 31 ± 4 years old) healthy donors. Diagnosis was performed by one of us (PvdL), who had no access to the biochemical data. Blood samples (five ml) were collected at least three times in a period of 15 months. Cell separation, measurement of erythrocyte-bound IgG, determination of anion transport characteristics, and immunoblot analysis of erythrocyte AE1 were performed in batches of eight individuals as described before (Bosman et al., 1991, 1993).

2. Materials and methods

2.1. Erythrocyte-bound Immunoglobulin G

The mean IgG values of all patient groups and of the age-matched, elderly control group were significantly higher than that of the young control donors.
(Table 1), confirming a previous observation that the erythrocyte IgG content is increased in the elderly (Bosman et al., 1993). At the start of the study, the mean erythrocyte-bound IgG values of the patient groups did not differ significantly between the groups, or between any of the patient groups and the age-matched controls (Table 1). After approximately 15 months after admission, however, the mean erythrocyte IgG content significantly increased in the groups with vascular dementia and mixed type dementia relative to the control groups (Table 1).

In contrast to the age-matched and young control groups, all patient groups showed a considerable individual variation in erythrocyte IgG content with time (Fig. 1). The percentage of individuals showing an arbitrary difference of 1.0 fgm/1000 erythrocytes between two subsequent measurements among the patients with a vascular component is twice that found in the other patient groups (80 and 75% in the VASC and MIX group, respectively, versus 40% in the SDAT group). There were no significant differences in the mean values of the slopes of the regression lines of the IgG values between the patient groups. However, in the SDAT group 55% of the patients had a negative slope, whereas in the vascular and mixed type dementia group the majority of the patients (65 and 75%, respectively) had a positive slope. There was no significant correlation between individual IgG values and GDS scores.

### 3. Anion transport characteristics

The mean values of the anion transport characteristics $K_m$ and $V_{\text{max}}$ (Bosman et al., 1991) were not significantly different from previous measurements, nor did they differ significantly between the various groups. However, they showed the same strong longitudinal variation as observed for the IgG concentrations. A negative correlation was observed between the values of IgG and of the $V_{\text{max}}$ of sulfate exchange, in agreement with previous findings (data not shown; Bosman et al., 1993).

<table>
<thead>
<tr>
<th>Group</th>
<th>3 months</th>
<th>9 months</th>
<th>15 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>AMC</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.4</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>SDAT</td>
<td>2.7 ± 1.7</td>
<td>2.7 ± 0.7</td>
<td>2.8 ± 2.4</td>
</tr>
<tr>
<td>ATY</td>
<td>2.1 ± 0.7</td>
<td>2.4 ± 1.3</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>VASC</td>
<td>3.3 ± 2.7</td>
<td>2.9 ± 0.6</td>
<td>4.0 ± 2.0*</td>
</tr>
<tr>
<td>MIX</td>
<td>2.4 ± 0.9</td>
<td>3.0 ± 2.0</td>
<td>5.5 ± 2.3*</td>
</tr>
</tbody>
</table>

* The amount of erythrocyte-bound IgG was measured as described before (Bosman et al., 1991), and is expressed in fgm/1000 erythrocytes. YC, young controls ($N = 6$); AMC, age-matched controls ($N = 10$); SDAT, senile dementia of the Alzheimer type ($N = 14$); VASC, vascular dementia ($N = 9$); MIX, mixed type dementia ($N = 5$); ATY, atypical dementia ($N = 7$). See the Section 2 for diagnosis and description of the donor groups. The mean group values were compared using 'one way analysis of variance', and values were considered significantly different (*) from the AMC and YC values when $P < 0.05$. 
4. Band 3 immunoblotting analysis

Immunoblot analysis of erythrocyte membranes with most anti-band 3 antisera shows, in addition to the major band 3 protein of 95 kDa, multiple smaller immunoreactive polypeptides (Fig. 2), that are probably breakdown products of band 3 (Kay, 1993). In SDAT patients fragmentation was already obvious at the
start of the study (Lane 1a), without a notable increase after one year (Lane 1b), whereas in most VASC patients fragmentation increases with time (Lane 2a, blood collected at the start of the study, with Lane 2b, blood collected 1 year later). Such an increase with time was not detectable in samples from age-matched control donors (Lanes 3a and 3b). The immunoblot of the MIX patients’ erythrocytes show band 3 patterns that are similar to those of the SDAT patients (Lanes 4a and 4b). Quantification of the band 3 breakdown products did not show a significant correlation between their relative amounts and any of the other aging-related parameters.

The results of this study confirm previous conclusions that erythrocyte membrane characteristics indicate a disturbance of the erythrocyte aging process in patients with dementia (Bosman et al., 1991, 1993). These previous data, however, were obtained in patients that were in a more advanced stage of the disease, had a longer duration of the disease, and had been studied much longer after their admittance to the psychogeriatric institution than the patients described here (Bosman et al., 1991). This comparison suggests that an increase in erythrocyte IgG content and changes in anion transport characteristics are not specific for Alzheimer-type dementia, and that they are only observed in advanced disease stages. Band 3 breakdown, however, is already significantly increased in patients with beginning stages of Alzheimer-type dementia, as was indicated by data obtained in individuals with Down’s syndrome (Bosman et al., 1993). A detailed immunochemical and structural analysis of the breakdown products should provide the means to further investigate this issue.

Fig. 2. Immunoblot analysis of band 3 fragmentation in erythrocyte membranes of patients with dementia. Section 1, SDAT; Section 2, VASC, Section 3, AMC; Section 4, MIX. Lanes a and b, erythrocytes collected at 3 and 15 months after admission to the psychogeriatric institution, or the start of the study (for AMC), respectively. For abbreviations see the legend to Table 1.
A complicating factor, reported here for the first time, is the variation with time in the erythrocyte aging characteristics of the demented elderly. None of the patients and control donors had any observable or measurable symptoms of physical illness at the times their blood samples were taken. This variation is therefore probably caused by variations in erythrocyte genesis and/or removal since, in the elderly, erythrocyte homeostasis is easily disturbed by subclinical stressors (Rothstein, 1993). This is supported by the observation that the erythrocyte IgG content increases with time especially in those patients with vascular dementia (VASC and MIX). These patients have more comorbidity, especially of cardiovascular nature, and a shorter life expectancy than SDAT patients (Koopmans, 1994; Wolf-Klein et al., 1988). Erythrocyte aging-related parameters such as erythrocyte IgG content may thus be sensitive biomarkers for disturbed systemic homeostasis in the elderly.

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References