Serum NGAL is Associated with Distinct Plasma Amyloid-β Peptides According to the Clinical Diagnosis of Dementia in Down Syndrome

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Published in
Journal of Alzheimer's Disease (IOS Press), 2015, 45: 733-743
Abstract

Background: The majority of people with Down syndrome (DS) develop dementia due to Alzheimer’s disease (AD). Neuropathological features are characterized by an accumulation of amyloid-β (Aβ) deposits and the presence of an activated immune response. Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a newly identified (neuro)inflammatory constituent in AD. Objective: This study examines NGAL as an inflammatory marker in DS and its associations with plasma Aβ peptides according to the follow-up clinical diagnosis of dementia. Methods: Baseline serum NGAL and plasma Aβ40, Aβ42, Aβn40, Aβn42 were quantified in 204 people with DS. The diagnosis of dementia in DS was established by follow-up clinical assessments. The following study groups were characterized: DS with AD at baseline (n=67), DS without AD (n=53) and non-demented DS individuals that converted to AD (n=84). Serum NGAL was analysed in 55 elderly non-DS, non-demented people. Results: Serum NGAL levels were significantly increased in DS subjects compared to non-DS people. Serum NGAL levels were not associated with clinical dementia symptoms in DS. However, NGAL was positively associated with Aβ42 and Aβn42 in demented DS individuals and with Aβ40 and Aβn40 in the non-demented DS group. NGAL was negatively associated with Aβ42:Aβ40 and Aβn42:Aβn40 ratios in converted DS subjects. These associations persisted for Aβn40, Aβ42:Aβ40, and Aβn42:Aβn40 after adjusting for demographics measures, Apolipoprotein E (ApoE) ε4 allele, platelets and anti-inflamatory medication. Conclusion: Serum NGAL levels are increased in DS and associated with distinct species of Aβ depending on the progression of dementia as diagnosed by baseline and follow-up clinical assessments.
8.1. Introduction

The prevalence of Down syndrome (DS), or trisomy 21, is approximately 1 in 700-1200 live births (Presson et al., 2013; Shin et al., 2009) and is the most common genetic incidence of intellectual disability in humans (Yang et al., 2002). A vast majority of people with DS develop Alzheimer’s disease (AD) pathology which is mainly characterized by amyloid-β (Aβ) depositions in the brain (Webb and Murphy, 2012). A high prevalence of the clinical diagnosis of dementia (50-70%) in DS is respectively found in mid- to late life (Mann, 1988; Zigman and Lott, 2007). This phenomenon is due to a triplication of the human chromosome 21 (HSA21) that harbors several genes, i.e. amyloid precursor protein (APP) and β-site APP cleaving enzyme 2, that are responsible for the increased production of Aβ (Webb and Murphy, 2012). In addition to increased brain Aβ levels, individuals with DS have increased plasma Aβ levels compared to people without DS (Mehta et al., 1998; Schupf et al., 2001; Tokuda et al., 1997).

Inflammatory-associated genes on HSA21 are likely overexpressed in DS and have been suggested to contribute to an aberrant immune regulation that is characterized by a pro-inflammatory environment (Wilcock, 2012; Wilcock and Griffin, 2013). Increased pro-inflammatory cytokines have been identified in brain tissue of people with DS (Griffin et al., 1989) as well as in their circulation (Rodrigues et al., 2014; Trotta et al., 2011), which might be even present during their early adolescence (Nateghi Rostami et al., 2012). Furthermore, increased neuroinflammatory processes have been suggested to play an important role in the pathophysiological processes of DS and AD (Wilcock, 2012; Wilcock and Griffin, 2013). This study focuses on Neutrophil Gelatinase-Associated Lipocalin (NGAL), a newly introduced inflammatory constituent in the pathophysiology of AD (Naudé et al., 2012). NGAL is a 25 kDa acute phase protein that is also known as Lipocalin-2, Siderocalin, 24p3, or Uterocalin (Kjeldsen et al., 1993). Human studies showed that increased blood NGAL levels are associated with risk factors for AD: mild cognitive impairment (Choi et al., 2011), late-life depression (Naudé et al., 2013), and elderly depressed females with impaired recall memory (Naudé et al., 2014). Serum NGAL is also increased in adult and elderly DS people compared to adult people without DS (Dogliotti et al., 2010). Primary neuronal cell cultures studies showed that NGAL mRNA and protein production is increased by Aβ_{42} (Mesquita et al., 2014) and Aβ_{40} (Paratore et al., 2006). Furthermore, NGAL impairs neuroprotective mechanisms in neurons and exacerbates Aβ_{42}-mediated neuronal cell death (Mesquita et al., 2014; Naudé et al., 2012). These studies in essence indicate that NGAL is an important inflammatory marker that is involved in the pathophysiology of AD.

The aims of this study were 1) to validate if NGAL levels are elevated in DS individuals compared to non-DS controls, 2) to determine whether baseline serum NGAL levels are associated with the clinical diagnosis of dementia in DS, i.e. DS subjects with established AD at baseline (demented), without AD (non-demented) and non-demented DS people that converted to dementia over time, and 3) to associate serum NGAL with plasma Aβ_{40}, Aβ_{42}, Aβ_{42}:Aβ_{40}, Aβ_{n40}, Aβ_{n42} or Aβ_{n42}:Aβ_{n40} in these groups.
8.2. Materials and methods

Study population

In total, 204 people with DS were included in this study. All participants were enrolled between 1 December 1999 and 1 December 2003 at an age of 45 years or older and are part of the previously published Rotterdam DS cohort (Coppus et al., 2012, 2008, 2007; Dekker et al., 2015). Fasting venous blood samples were obtained in the morning, once at baseline of the study. Blood was directly processed and plasma and serum were stored at -80°C and -20°C, respectively. Ethical approval for this study was granted by the ethical review board of Erasmus MC Rotterdam (METc protocol no.: MEC 185.974/1999/202). Written informed consent to participate and to provide blood samples was obtained from legal representatives (relatives and/or caretakers), after written information was provided. Written consent was also obtained from persons with DS who had the mental capacity to consent. To determine whether NGAL levels are increased in DS compared to healthy non-DS persons, serum samples from 55 healthy non-DS persons were obtained from the Antwerp Biobank of the Institute Born-Bunge. These volunteers did not have any illness, clinical variables nor did they use any medication which may have interfered with NGAL levels. Ethics approval for human sample collection of serum was granted by the Medical Ethical Committee of the Middelheim General Hospital (Antwerp, Belgium) (Approval no. 2805 and 2806). The study was also conducted in compliance with the Helsinki Declaration.

Clinical AD assessment

As previously described (Coppus et al., 2006; Dekker et al., 2015), AD was assessed at baseline using the International Classification of Diseases (ICD)-10 from the World Health Organization, according to the guidelines of the Special Interest Research Group on Aging of the International Association for the Scientific Study of Intellectual Disabilities (IASSID) to diagnose dementia in adults with intellectual disabilities (Aylward et al., 1997; Burt and Aylward, 2000; World Health Organization, 2010). These criteria emphasize on non-cognitive symptoms, which are often prominent signs of dementia in adults with intellectual disabilities. Importantly, ICD-10 criteria have been modified for use in adults with intellectual disabilities. It has been shown that the AD criteria of the ICD-10 and the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) diagnosed dementia in the same adults with DS (Holland et al., 1998) and that these diagnostic criteria show ‘substantial reliability and satisfactory validity’ in other intellectual disabilities as well (Strydom et al., 2013).

In our study, study participants were systematically screened for dementia and examined in person by a clinician. The demented individuals met the ICD-10 criteria at intake and had an insidious and progressive course of the disease. In addition, validated functional questionnaires such as the Dementia Questionnaire for persons with an intellectual disability (DMR) (Evenhuis et al., 1998), Social Competence Rating Scale for persons with an intellectual disability (SRZ) (Kraijer et al., 2004), and, Vineland adaptive behaviour scales (Sparrow and Cicchetti, 1985) were prospectively completed by family or caretakers every twelve months (continues until present if the person is still alive). Three diagnostic groups were defined based on the AD assessment (ICD-10) and annual follow-
up (DMR, SRZ, and Vineland): demented at baseline (n=67), converted (n=84) and non-
demented (n=53) DS subjects. DS people that converted to AD, was clinically established
before January 2007, thus within 3 to 7 follow-up years after intake and blood sampling.
All of the DS participants in this study were assessed annually from baseline until January
2013 and were therefore followed for 10-14 years since baseline of this study. Body Mass
Index (BMI) at baseline was computed as weight in kilograms divided by height in square
meters.

**Analyses of blood samples**
Blinded analysis of serum NGAL (Naudé et al., 2012), plasma Aβ_{40}, Aβ_{42}, and truncated
Aβ_{n40}, and Aβ_{n42} (Coppus et al., 2012) and Apolipoprotein E (ApoE) genotype (Coppus et
al., 2008) was performed as previously described. Blood (20 ml) obtained via the
antecubital vein was collected in tubes containing K_2-EDTA and immediately processed for
platelet preparation. Platelet-rich plasma and blood cell fractions were separated by
centrifugation. Platelet-rich plasma was removed and centrifuged again to obtain platelet
pellets. Platelets were suspended in sucrose containing 5% dimethylsulfoxide to maintain
membrane integrity and stored at −80 °C until use.

**Covariates**
Age, gender and BMI were included as covariates based on previous findings (Naudé et al.,
2013). The presence of the ApoE ε4 allele was included as covariate as well since it can
affect serum inflammatory markers (Zhao et al., 2012) and possibly plasma Aβ levels
(Toledo et al., 2011). Furthermore, blood platelets were included as final confounding
factor, since previous studies described them as an importance source of plasma Aβ_{40} and
Aβ_{42} (Chen et al., 1995; Roher et al., 2009). Recently, in a large cohort with elderly
participants we showed that increased NGAL levels were associated with the use of anti-
inflammatory medication (Naudé et al., 2013). Therefore, the use of non-steroidal anti-
inflammatory drugs (NSAIDs) was included as final covariate. Only three DS people used
corticosteroids and they were therefore not included as covariate.

**Statistical analyses**
In order to obtain a normal distribution of the serum NGAL levels, four identified outliers
were trimmed to 304.19 ng/ml resulting in a skewness of 0.65 and kurtosis of -0.25. As
some covariates had missing data, we imputed the mean value of the other subjects in
case of continuous variables or the most frequent score in case of dichotomous or
nominal data. Variables with missing values in the whole sample were: BMI (n=5), ApoE
(n=4), platelets (n= 9), Aβ_{40} (n=11), Aβ_{42} (n=10), Aβ_{42}:Aβ_{40} (n=11), Aβ_{n40}
(n=11), Aβ_{n42} (n=22), and Aβ_{n42}:Aβ_{n40} (n=22). Missing Aβ variables were due to insufficient plasma
volumes for the analyses of Aβ peptides. First, for the description statistics of study
participant demographics, analysis of variance (ANOVA) was performed for continuous
variables (with a Tukey post-hoc test for pair-wise comparisons in case of an overall effect
between the three groups, i.e. age), and Pearson’s chi squared tests for categorical
variables. ANOVA with Tukey post hoc test for pair-wise comparisons was used to
determine differences of NGAL protein levels between non-DS controls, demented,
converted and non-demented DS people. This was followed by analyses of covariance (ANCOVA) with Bonferroni post hoc test with serum NGAL levels as dependent variable to analyse NGAL levels between the studied groups, adjusted for age and gender as confounding factors. First, ANCOVA was performed to determine the interaction of Aβ_{40}, Aβ_{42}, Aβ_{42:40}, Aβ_{n40}, Aβ_{n42} or Aβ_{n42:40} with diagnostic status of dementia (non-demented, converted and demented at baseline) with NGAL as the dependent variable. A P-value of less than 0.1 was considered as statistically significant for interaction terms (Durand, 2013). Since an interaction effect was found, linear regression analyses were conducted separately within each DS study group, with NGAL as the dependent variable, to examine its associations with serum Aβ_{40}, Aβ_{42}, Aβ_{42:40}, Aβ_{n40}, Aβ_{n42} or Aβ_{n42:40}. Subsequently, multiple regression models were performed separately for the three different DS groups, with NGAL as dependent variable, to examine the associations of plasma Aβ_{40}, Aβ_{42}, Aβ_{42:40}, Aβ_{n40}, Aβ_{n42} or Aβ_{n42:40} with serum NGAL concentrations adjusted for confounding variables. P-values for were considered statistically significant at a value of less than 0.05. All analyses were conducted with SPSS version 22.0.

8.3 Results

**Population demographics**

Demographics and clinical information of non-DS controls and DS persons are shown in Table 8.1. Non-DS controls were older than DS people and DS subjects with dementia at baseline and people whom converted to dementia during follow-up were older than the non-demented DS group. No significant differences were observed for gender, BMI, ApoE ε4 allele or platelet numbers between groups. Significant differences in NGAL levels were found between the non-DS people and the DS groups. While the presence of ApoE ε4 allele have been associated with increased blood pro-inflammatory cytokines in humans (Olgiati et al., 2010; Zhao et al., 2012), results from this study show that NGAL levels were not significantly associated with the presence of the ApoE ε4 allele (unpaired t-test, t(198)=0.416; P=0.321).

**Table 8.1: Demographics and clinical info of study participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-DS controls (n=55)</th>
<th>Demented DS (n=67)</th>
<th>Converted DS (n=84)</th>
<th>Non-demented DS (n=53)</th>
<th>Statistics for DS participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, female n</td>
<td>25 (46)</td>
<td>26 (39)</td>
<td>33 (39)</td>
<td>21 (40)</td>
<td>X^2=0.71, df=3,</td>
</tr>
<tr>
<td>Age (years), mean</td>
<td>75.5 (9.4) ^a</td>
<td>54.5 (5.9) ^b</td>
<td>53.1 (5.3) ^c</td>
<td>49.7 (4.3)</td>
<td>F(3, 255)=190.38,</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>25 (4.8)</td>
<td>25.7 (3.9)</td>
<td>25.4 (3.8)</td>
<td>F(2, 198)=0.41,</td>
<td></td>
</tr>
<tr>
<td>ApoE ε4 allele, n</td>
<td>22 (33.8)</td>
<td>21 (25.6)</td>
<td>14 (26.4)</td>
<td>X^2=1.36, df=2,</td>
<td></td>
</tr>
<tr>
<td>Platelets, mean</td>
<td>232.1 (78.4)</td>
<td>224.7 (90.9)</td>
<td>232.1 (73.6)</td>
<td>F(2, 192)=0.19,</td>
<td></td>
</tr>
<tr>
<td>NSAID, n (%)</td>
<td>11 (19)</td>
<td>10 (12.8)</td>
<td>3 (6.3)</td>
<td>X^2=4.40, df=2,</td>
<td></td>
</tr>
<tr>
<td>NGAL, mean (SD)</td>
<td>114.4 (52.2)</td>
<td>162.5 (37.5)</td>
<td>155.2 (53.6)</td>
<td>F(3, 255)=10.12,</td>
<td></td>
</tr>
</tbody>
</table>

^a Non-DS controls vs demented at baseline, converted and non-demented P<0.001
^b Demented vs non-demented P=0.001
^c AD converted vs non-demented P=0.013

Abbreviations: AD, Alzheimer’s disease; ApoE, Apolipoprotein E; BMI, body mass index; DS, Down syndrome; n, number; NGAL, neutrophil gelatinase-associated lipocalin; NSAID, non-steroidal anti-inflammatory drugs; SD, standard deviation.
Serum NGAL levels in healthy non-DS volunteers compared to DS individuals

Differences in NGAL levels between the studied groups was further explored, since significant differences in NGAL levels between non-DS controls, demented, converted and non-demented DS groups (ANOVA, F=10.12, df=3, P<0.001) were found. NGAL levels were significantly lower in non-DS individuals 114.35 (37.5) ng/ml compared to demented 162.5 (61.9) ng/ml (P<0.001) converted 155.2 (53.6) ng/ml (P<0.001) and non-demented DS 163 (63.7) ng/ml (P<0.001) people (Figure 8.1). Moreover, analysis with ANCOVA (F(3,253)=8.69, P<0.001) and Bonferroni post hoc tests showed that serum NGAL levels were increased in demented converted and non-demented compared to non-DS controls (P<0.001 in all groups) after including age and gender as covariates.

![Figure 8.1](image)

Association of NGAL with Aβ levels, characterized by diagnosis of dementia in DS

Firstly, ANCOVA analyses were performed to determine the interaction of Aβ_{40}, Aβ_{42}, Aβ_{42}:Aβ_{40}, Aβ_{n40}, Aβ_{n42}, or Aβ_{n40}:Aβ_{n40} with the diagnostic status of dementia (demented, converted and non-demented), with NGAL as dependent variable, to verify if associations of NGAL with Aβ should be performed separately in the DS groups based on dementia diagnosis. Outcomes from ANCOVA showed the following interactions; Aβ_{40} (P=0.838), Aβ_{42} (P=0.050), Aβ_{42}:Aβ_{40} (P=0.435), Aβ_{n40} (P=0.053), Aβ_{n42} (P=0.007) or Aβ_{n42}:Aβ_{n40} (P=0.071). Since the majority showed a significant interaction, as the P-value was considered significant less than 0.1, the presented results are stratified by dementia diagnosis. As shown in Table 8.2, higher serum NGAL levels were significantly associated with higher plasma Aβ_{40} levels in the non-demented DS group, which remained significant after adjustments for confounding factors; age, gender, BMI, ApoE ε4 and platelets, but

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the significant association was lost after including NSAIDs as confounding factor. Linear regression analyses showed a significant association of higher NGAL levels with higher Aβ_{42} levels. However, significance was lost after correcting for confounding factors. Higher NGAL levels were significantly associated with a lower Aβ_{42}:Aβ_{40} ratio in converted DS people. This association lost significance after adjusting for age, gender, BMI, ApoE ε4 and platelets, however remained significant after including NSAIDs as covariant. Higher NGAL showed a strong association with higher Aβ_{40} levels in non-demented DS people independent of confounding factors. A significant association of higher NGAL levels with higher Aβ_{42} levels was found in the demented DS group, which remained significant after correcting for age, gender, BMI, ApoE ε4 and platelets. Inclusion of NSAIDs as covariant consequently resulted in a significant association of increased NGAL levels with increased Aβ_{42} levels in the demented and non-demented DS individuals and decreased Aβ_{40} levels in the converted DS people. Increased NGAL levels were significantly associated with a decreased Aβ_{42}:Aβ_{40} ratio in converted DS subjects. This association remained marginally significant (P=0.055) after correcting for age, gender, BMI and ApoE ε4. Accordingly, the association remained significant after inclusion of platelet levels and NSAIDs.

8.4 Discussion
The current study shows that serum NGAL levels were increased in elderly DS subjects compared to healthy, non-DS controls. Furthermore, serum NGAL levels were not associated with the clinical symptoms of dementia in DS. However, definite associations of NGAL levels with Aβ_{40}, Aβ_{42}, their truncated species, and their ratios depended on the follow-up clinical diagnosis of dementia. Therefore, these results support the notion that a pro-inflammatory environment is present in DS and that NGAL is an inflammatory marker that is significantly associated with distinct species of Aβ, moderated by the presence or absence of the clinically established dementia diagnosis over time.

**Serum NGAL levels in DS and healthy non-DS subjects: the role of Aβ**
As mentioned above, our results show that serum NGAL levels in older DS people were significantly increased compared to healthy elderly non-DS people. This finding is in accordance with a study by Dogliotti and colleagues (2010) showing that serum NGAL levels are significantly increased in adults and elderly people with DS compared to adult non-DS healthy controls. Because NGAL is encoded on human chromosome 9 (Chakraborty et al., 2012), increased NGAL levels may not be directly attributed to the triplication of HSA21. Importantly, studies with neuronal cell cultures have shown that NGAL protein and mRNA production is stimulated by Aβ_{42} (Mesquita et al., 2014) and Aβ_{40} (Paratore et al., 2006). In this regard, a robust increase of NGAL protein levels is present in post-mortem brain tissue of AD patients with a similar regional distribution pattern as the Aβ pathology (Naudé et al., 2012). These studies, therefore, indicate that increased NGAL production may be the result of Aβ accumulation that is characteristically present in DS brain, already at a young age. NGAL thus may be related to Aβ-related pathophysiological processes in the development of dementia in DS. Correspondingly, the association of serum NGAL with different plasma Aβ species was further investigated in this study population.
Table 8.2: Association of serum NGAL levels with plasma Aβ species, including covariates, per diagnostic DS group

<table>
<thead>
<tr>
<th></th>
<th>Aβ_{40}</th>
<th>Aβ_{42}</th>
<th>Aβ_{42}:Aβ_{40}</th>
<th>Aβ_{n40}</th>
<th>Aβ_{n42}</th>
<th>Aβ_{n42}:Aβ_{n40}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B(SE)</td>
<td>β</td>
<td>P</td>
<td>B(SE)</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td><strong>Unadjusted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demented</td>
<td>.43(.23)</td>
<td>.24</td>
<td>.064</td>
<td>.30</td>
<td>.021</td>
<td>3.25(1.36)</td>
</tr>
<tr>
<td>Converted</td>
<td>.30(.16)</td>
<td>.21</td>
<td>.056</td>
<td>-1.09(1.23)</td>
<td>-.10</td>
<td>-.192.75(96.68)</td>
</tr>
<tr>
<td>Non-demented</td>
<td>.44(.21)</td>
<td>.28</td>
<td>.042</td>
<td>2.75(1.66)</td>
<td>.23</td>
<td>-103.80(108.33)</td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demented</td>
<td>.25(.25)</td>
<td>.14</td>
<td>.31</td>
<td>2.29(1.36)</td>
<td>.20</td>
<td>.097</td>
</tr>
<tr>
<td>Converted</td>
<td>.27(.16)</td>
<td>.20</td>
<td>.084</td>
<td>-1.04(1.29)</td>
<td>-.09</td>
<td>-.190.90(98.71)</td>
</tr>
<tr>
<td>Non-demented</td>
<td>.44(.22)</td>
<td>.28</td>
<td>.049</td>
<td>2.61(1.75)</td>
<td>.21</td>
<td>.14</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demented</td>
<td>.23(.24)</td>
<td>.13</td>
<td>.96</td>
<td>2.13(1.36)</td>
<td>.19</td>
<td>.12</td>
</tr>
<tr>
<td>Converted</td>
<td>.21(.16)</td>
<td>.16</td>
<td>.19</td>
<td>-1.40(1.29)</td>
<td>-.13</td>
<td>-.175.60(99.20)</td>
</tr>
<tr>
<td>Non-demented</td>
<td>.45(.22)</td>
<td>.29</td>
<td>.049</td>
<td>2.64(1.81)</td>
<td>.21</td>
<td>.15</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demented</td>
<td>.24(.25)</td>
<td>.15</td>
<td>.35</td>
<td>2.14(1.37)</td>
<td>.22</td>
<td>.13</td>
</tr>
<tr>
<td>Converted</td>
<td>.19(.17)</td>
<td>.14</td>
<td>.26</td>
<td>-2.08(1.35)</td>
<td>-.19</td>
<td>-.211.36(101.89)</td>
</tr>
<tr>
<td>Non-demented</td>
<td>.44(.23)</td>
<td>.29</td>
<td>.062</td>
<td>3.81(1.92)</td>
<td>.31</td>
<td>.055</td>
</tr>
</tbody>
</table>

Model 1: Adjusted for age, gender, BMI, and ApoE e4 allele.
Model 2: Model 1, added with platelet levels.
Model 3: Model 1 and 2, added with use of NSAID.
Abbreviations: Aβ, amyloid-β; BMI, body mass index; DS, Down syndrome; NGAL, neutrophil gelatinase-associated lipocalin; NSAID, non-steroidal anti-inflammatory drugs.
The bold values indicate significant associations.
Associations between serum NGAL levels and different plasma Aβ species

Increased serum NGAL levels were 1) positively associated with Aβ42 and Aβn42 in the demented DS group, 2) positively associated with Aβ40 and Aβn40 in non-demented DS subjects, and 3) negatively associated with Aβ42:Aβ40 and Aβn42:Aβn40 ratios in those non-demented DS individuals that converted to dementia over time. These findings are of interest considering the neuropathological regulation of Aβ accumulation in DS during lifetime. Neuropathological studies in DS demonstrated that sequential changes of Aβ plaque formation occur during the lifespan in people with DS, which can provide insights concerning the associations of NGAL with Aβ found in this study. Intraneural Aβ42, but not Aβ40, has been reported in very young DS people (3 years old) (Mori et al., 2002). With increasing age, extracellular Aβ42 plaques gradually accumulate and mature (Iwatsubo et al., 1995; Mori et al., 2002). Extracellular deposition of Aβ42 in senile plaques precedes the presence of Aβ40 by approximately a decade (Iwatsubo et al., 1995; Teller et al., 1996). During the later stages in life (around 50 years), Aβ40 accumulation gradually increases in mature plaques and, moreover, it is the predominant Aβ species in cerebral amyloid angiopathy in DS (Iwatsubo et al., 1995; Lemere et al., 1996). Although almost all individuals with DS have Aβ deposition resembling AD neuropathology (Mann and Esiri, 1989; Wisniewski et al., 1985), there is a wide variation in the age at onset of dementia. This is due to complex mechanisms that are involved in Aβ regulation during the progression to dementia (Dekker et al., 2014). In this respect, alterations in the ratio between Aβ42 and Aβ40 may function as a significant predictor for the development of dementia due to AD (Koyama et al., 2012; Selkoe, 2001).

The positive association of increased NGAL with Aβ40 in non-demented DS subjects may indicate that Aβ40 has not yet accumulated into plaques in the brain resulting in a positive correlation with NGAL in the peripheral blood circulation. This association remained significant after adjustments for confounding factors were made. On the other hand, the association of increased NGAL with Aβ42 in the demented DS group may be explained by microglial processes during later stages of Aβ pathology in DS. It was shown that activated microglia and astrocytes were present in diffuse and neuritic plaques (Gyure et al., 2001) and microglia cells can clear Aβ42 from the brain to compensate for Aβ pathology (Perlmuter et al., 1992). Alternatively, increased inflammatory processes associated with microglia activation may induce an increase in APP and consequently an increase in Aβ42 production (Wilcock, 2012). Both of these abovementioned processes can lead to increased levels of circulating Aβ42 peptides. However, this significant association diminished after adjustments for age, gender, BMI and ApoE ε4 allele. Interestingly, increased NGAL levels were negatively associated with the Aβ42:Aβ40 ratio in the converted DS group. This association remained marginally significant after the adjustments for age, gender, BMI and ApoE ε4 were made. Considering changes of Aβ40 and Aβ42 in the brain described in the abovementioned neuropathological studies and the association of increased serum NGAL with plasma Aβ40 in non-demented and Aβ42 in demented DS subjects, it is reasonable to speculate that NGAL is associated with a shift in Aβ regulation present in people with DS whom are in process of converting to dementia. Moreover, it has been previously shown that truncated Aβ increases in parallel to their full length.
peptides in DS brain (Russo et al., 1997). Similar associations of NGAL with full length Aβ and their truncated isoforms can therefore be expected. Indeed, our findings persisted for Aβ₄₀ and Aβ₄₂, similarly to their full-length isoforms. Generally, the association of NGAL levels was even stronger with truncated forms of Aβ than with full length Aβ.

The association of NGAL levels with Aβ₄₂:Aβ₄₀ and Aβ₄₀:Aβ₄₂ ratio strengthened after adjusting for NSAIDs as confounding factor. In addition, the associations of NGAL levels with Aβ₄₂ levels became significant in all of the DS groups. In a previous cohort with a large population of elderly participants, we found that increased NGAL levels were associated with the use of anti-inflammatory medication, which may be explained by underlying somatic conditions (Naudé et al., 2013). Therefore, the increase in significance of associations after correcting for NSAIDs may be due to correcting for underlying physical ailments related to inflammatory conditions, explaining additional variance in NGAL levels unrelated to levels of Aβ peptides.

The relationship between NGAL, neurodegeneration and DS

Fundamental research indicates that NGAL plays a role in several mechanisms involved in the pathophysiology of AD. Cell culture studies have shown that NGAL induces pro-apoptotic signaling cascades in neurons and exacerbates oligomeric Aβ₄₂-mediated neuronal cell death (Mesquita et al., 2014; Naudé et al., 2012). In addition, NGAL can aggravate oxidative damage to neuronal cells (Lee et al., 2009; Mesquita et al., 2014). This is of importance since people with DS have an increased susceptibility for oxidative stress due to an extra copy of superoxide dismutase 1 (SOD1) (Zigman and Lott, 2007). Furthermore, NGAL exerts neuro-immunomodulatory effects. Increased NGAL induces astrocytes and microglia to a pro-inflammatory phenotype and silences their anti-inflammatory functioning (Jang et al., 2013a, 2013b), whereas elimination of NGAL reduced neuroinflammation and neuronal damage after neuronal injury in mice (Jin et al., 2014; Rathore et al., 2011). As basal NGAL levels increase with age in DS (Dogliotti et al., 2010), it could increase the sensitivity towards toxic forms of Aβ and oxidative stress and, therefore, contribute to neurodegeneration and, consequently, the development of clinical symptoms of dementia that occur mid- to late life in DS.

Plasma Aβ as a potential biomarker for dementia conversion in DS

Blood based biomarkers that can predict the conversion to dementia in DS are much desired because they would provide a valuable tool to enable and plan optimal adaptive caregiving. In addition, biomarkers can improve our knowledge of aberrant physiological processes involved during the disease progression. Several studies have investigated the association of plasma Aβ in DS and their potential as diagnostic markers for dementia with inconsistent results (Toledo et al., 2013). A possible explanation for these discrepancies is that changes of plasma Aβ concentrations in relation to the status of dementia might not be large enough for its use as a biomarker. In this respect, results from this study indicate that the association of NGAL with Aβ species may provide an indication of changes in Aβ accumulation during the progression to dementia in DS.
Strengths and limitations

This study has several strengths worth mentioning. This study consisted out of a large DS population group. In addition to AD diagnosis at baseline using the ICD-10 criteria, follow-up clinical assessment in this DS population using validated questionnaires for dementia in DS enabled the identification of those DS individuals that remained non-demented or converted to dementia over time. Several important confounding factors were included that were shown to have potential associations with NGAL and Aβ. The role of circadian influences on blood markers was minimized by obtaining fasting morning blood samples. In addition, NGAL possesses great storage stability i.e. NGAL can be subjected to several freeze-thaw cycles without affecting outcomes of its analyses which make it suitable for application as a biomarker (Pedersen et al., 2010).

In order to properly interpret the results presented in this study, study limitations ought to be acknowledged. ANCOVA analysis did not show a significant interaction of Aβ40 and Aβ42:Aβ40 with the diagnosis of dementia, with NGAL as dependent variable and therefore, outcomes from these findings should be interpreted with caution. Increased significant associations of NGAL levels with Aβ42:Aβ40 and Aβ40:Aβ42 ratio and Aβn42 after correcting for NSAIDs may be due to underlying ailments that were not documented in this study. Results of this study are based on baseline blood sampling, but longitudinal studies with clinical assessments of dementia in DS accompanied with follow-up blood collection is warranted. Of particular interest would be to follow DS people from a younger age (<40 years) to accurately evaluate the association of NGAL with Aβ in the progression to dementia.

8.4. Conclusion

In conclusion, this study confirmed that serum NGAL levels are increased in elderly DS subjects compared to elderly non-DS controls and strengthens the notion that an increased pro-inflammatory condition is present in people with DS. Furthermore, NGAL was not associated with either diagnosed dementia or progression to dementia in DS. However, serum NGAL levels were associated with different plasma Aβ species according to the clinical symptoms of dementia. Therefore, the association of serum NGAL with plasma Aβ may reflect the neuropathological regulation of Aβ accumulation and circulation in accordance to the clinical symptoms of dementia in DS. Finally, the measurement of circulating NGAL levels may improve the sensitivity of plasma Aβ as a biological marker for dementia in DS that merits further investigation.

References
