Erythropoietin in anemia of unknown etiology: A systematic review and meta-analysis

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Introduction: We conducted a systematic review and meta-analysis of observational studies in order to explore the relationship between erythropoietin (EPO) and hemoglobin in elderly individuals with anemia of unknown etiology (AUE) and other forms of anemia.

Methods: We searched Medline, EMBASE, Web of Science, Biosis Previews, Dissertations, and Theses in addition to meeting abstracts of the European Hematology Association and American Society of Hematology for relevant studies. The meta-analysis was conducted using pooled ratio of means (ROM) through the generic inverse variance method.

Results: Six studies were included in the meta-analysis, which confirmed that EPO levels were significantly lower in AUE as compared to iron deficiency anemia (ROM 0.7210; random 95% CI 0.7052 to 0.7372; P-value < 0.00001) and anemia of chronic disease (ROM 0.8995; random 95% CI 0.8362 to 0.9677; P = 0.004). EPO levels in AUE were slightly higher than levels in anemia of chronic kidney disease (ROM 1.0940; random 95% CI 1.0557, 1.1337; P < 0.00001). The heterogeneity (I²) of all analyses was 100%.

Conclusion: Our findings suggest that erythropoietin levels in AUE, although elevated, remain inappropriately low, particularly when compared with other forms of anemia. This suggests a relative erythropoietin deficiency or a blunted erythroid cell response.

Keywords: Aged, Geriatric, Anemia, Erythropoietin, Elderly

Introduction
Anemia is a significant issue in the geriatric population, having an important impact on the functioning of elderly individuals. It is an independent predictor of mortality⁴ and is associated with significant morbidity, including poor physical performance, increased susceptibility to falling, and impaired cognition. As per the World Health Organization criteria, the diagnosis of anemia is made in men when the hemoglobin is less than 130 g/L and in women when the hemoglobin is less than 120 g/L. In nursing home residents, the prevalence of anemia is approximately 20–40%,⁵ while in the remainder of the community, the prevalence of anemia is approximately 10%.⁶ However, despite extensive investigations, approximately one-third of the anemic elderly population does not have a clearly identifiable cause for the anemia.⁵–⁷ In a study evaluating the etiology of anemia in an outpatient population of patients aged 65 years and older, anemia of unknown etiology (AUE) accounted for 43.7% of the patients diagnosed with anemia.⁶

There are several postulated mechanisms of AUE including a blunted response to erythropoietin (EPO).⁸ It has been suggested that elevated levels of inflammatory cytokines blunt the response of hematopoietic progenitor cells to stimulating factors, which includes EPO. Although elevated levels of inflammatory cytokines are often the result of a biological stress, this regulation becomes less precise with aging.⁸ Furthermore, there is an age-related decline in the ability of hematopoietic stem cells to maintain appropriate proliferative homeostasis; this is also a likely contributor to AUE in the elderly population.⁹ Lastly, inadequate EPO production in response to anemia has also been suggested as a possible mechanism for AUE. Studies have found that EPO levels are lower than expected (even in the absence of evidence of inflammation) in AUE.⁵–¹¹

The latter mechanism is of particular interest since it might be amenable to pharmacological intervention. Therefore, in order to explore the relationship between EPO levels and hemoglobin in elderly
individuals with AUE, we conducted a systematic review and meta-analysis of observational studies exploring EPO levels in elderly anemia patients.

Methods

Literature search

We aimed to include any study evaluating or potentially evaluating EPO levels in elderly patients with anemia. In order to be included, studies had to report data on EPO levels in AUE in the elderly or geriatric population with comparisons being made to other causes of anemia such as anemia of chronic disease (ACD) and renal dysfunction. In all studies, AUE was a diagnosis of exclusion if all other investigations for anemia were negative. The search was conducted in the following databases: Medline, EMBASE, Web of Science, Biosis Previews, and Dissertations, and Theses. We used the MeSH subject headings ‘erythropoietin’, ‘anemia’, ‘elderly’ and ‘diagnosis’ which were combined using Boolean operators. Additional relevant articles were identified by hand searching the abstract handbooks of the meetings of the European Hematology Association (2006–2012) and the American Society of Hematology (2004–2012). All articles were evaluated for inclusion by a single reviewer and confirmed by a second reviewer. Discrepancies were resolved by consensus. The quality of the studies included in the systematic review and meta-analysis was assessed and defined according to the Newcastle-Ottawa Quality Scale.12

Statistical analysis

A meta-analysis was conducted in studies with available data. In order to estimate the EPO levels across different groups, we calculated the ratio of means (RoM) of the EPO levels in AUE versus a comparison group. This method allows for comparisons across studies evaluating the same analyte but using different reagents, instruments, or reference ranges. This method has been validated and shown to exhibit a performance comparable to more traditional measurements such as mean difference or standardized mean difference.13 The RoM can be interpreted as an odds ratio in terms of analyzing and comparing differences between the EPO levels.

Briefly, the RoM for the serum EPO level was calculated for each type of anemia as follows: $RoM = \frac{\text{mean}_{\text{AUE}}}{\text{mean}_{\text{control}}}$, with the ‘control’ representing either iron deficiency anemia (IDA), ACD, or anemia of chronic kidney disease (CKD). Since studies included in the meta-analysis did not consistently report standard errors or standard deviations, the latter was estimated from ranges or inter-quartile ranges. Variance was estimated using the delta method.14 Standard error was calculated as the square root of variance divided by sample size. Finally, the ratio of means was log-transformed and the results were pooled using the generic inverse variance method using a random effects model. The pooled estimates were back transformed and reported as a pooled ratio of means (RoM) with a 95% CI. Statistical heterogeneity was assessed using Higgins’ $I^2$ test and categorized as low if $<25\%$, intermediate if 25–75% and high if $>75\%$.

The analyses were done using Excel (Microsoft Corp., Redmond WA, USA) and Review Manager v5.2.15

Results

Search results and characteristics of the included studies

The search identified 4277 potentially relevant citations of which 31 studies were reviewed in full; 7 were included in the systematic review (Table 1) and 6 in the meta-analysis. The flow of the search is shown in Fig. 1.

The quality of each cohort study was assessed according to the Newcastle-Ottawa Quality Scale.12 With the exception of the study by Kario et al.,16 all studies were rated as three stars with regard to selection of the cohort and one star for comparability of the control population.

All of the identified studies were cohorts – three prospective and four retrospective. In five of the seven studies, EPO levels in AUE were compared to other subtypes of anemia in addition to healthy, non-anemic (usually) elderly controls. Three studies included data on anemia resulting from vitamin B$_{12}$/folate deficiency,6,17,18 and five studies included data on ACD.4,6,7,16,18

The seven studies included in the systematic review involved 2534 individuals of which 1237 were diagnosed with anemia. Of those diagnosed with anemia, 536 of these individuals were male (40.0%). The median age of individuals ranged from 74 to 88. The majority of studies,6,11,16,18 included individuals greater than 60 years of age. All of the individuals in study by den Elzen et al.17 were 86 years of age. The range of ages in the study by Artz et al.5 was quite broad, with participants ranging in age from 49 to 94. Of the individuals diagnosed with anemia, 458 patients (46.3%) had AUE, 181 patients (18.3%) were diagnosed with IDA, 26 (2.6%) were diagnosed with vitamin B$_{12}$/folate deficiency, 91 (9.2%) of patients had anemia attributed to inflammation/chronic disease and 71 patients (2.8%) were diagnosed with anemia associated with CKD or hemodialysis. The remainder of patients had anemia that was attributed to other causes, including hematologic malignancies.
| Author, year       | Study design  | n   | Mean age (years) | AUE (n/%) | IDA (n/%) | Anemia associated with CKD or HD (n/%) | ACD (n/%) | EPO levels in AUE | Summary of findings                                                                 |
|-------------------|---------------|-----|------------------|-----------|-----------|----------------------------------------|-----------|------------------|-----------------------------------------------------------------------------------|
| Artz et al. (2004) | Retrospective cohort | 60  | 82 (range 49–94) | 27 (45%)  | 14 (23%)  | 6 (210%)                               | 8 (13.3%) | 14.6 mIU/mL ± 7.3 | Significant difference in EPO levels between IDA and AUE (P = 0.003) Univariate analysis of AUE: no correlation between EPO and Hb concentrations |
| Artz and Thirman (2011) | Prospective cohort | 174 | 76 (all subjects ≥65) | 76 (43.7%) | 44 (25.3%) | 6 (3.4%)                               | 17 (9.8%) | 14 mIU/mL (7.6–31) | Significantly lower EPO levels in AUE compared to IDA (P < 0.0001) Log-transformed EPO levels: no correlation with Hb in AUE |
| den Elzen et al. (2013) | Prospective cohort | 490 | 86 (all participants aged 86) | 29 (5.91%) | 13 (2.65%) | 8 (1.63%)                               | 23 (20.2%) | 11.1 mIU/mL (7.5–13.9) | Lower EPO levels in AUE (11.1 mIU/mL) compared to IDA (16.2 mIU/mL) |
| Ferrucci et al. (2007) | Prospective cohort | 964 | 81 (SD 8) | 42 (36.8%) | 19 (16.7%) | 9 (7.9%)                               | 32 (28.1%) | 7.7 mIU/mL | Significantly lower EPO levels in AUE compared to non-anemic controls AUE: little EPO compensation with low Hb levels |
| Kario et al. (1992) | Prospective cohort | 247 | 78 (SD 8) | 65 (67.0%) | 8 (8.25%) | N/A                                    | N/A       | 29.5 mIU/mL (20.8–41.6) | EPO levels were highest in IDA EPO in AUE slightly higher than non-anemic controls No correlation seen between EPO and Hb in AUE |
| Price et al. (2011) | Prospective cohort | 190 | 77.8 (SD 7.2) | 67 (35%)  | 23 (12%)  | 8 (4%)                                 | 11 (6%)   | Not available | Quantile regression model: trend of higher EPO levels in IDA compared to AUE at an Hb of 10 g/dL |
| Waalen et al. (2011) | Retrospective cohort | 409 | 74.0 (SD 5.5) | 152 (61.8%) | 60 (24.4%) | 34 (13.8%)                             | N/A       | Men: 10.0 mIU/mL ± 7.0 Women: 8.6 mIU/mL ± 4.0 | Significantly higher EPO levels in AUE compared to matched controls, but modest compared to EPO levels in IDA AUE: no correlation between EPO and Hb concentration |

*aAge of the AUE population only.

*bThe percentage is expressed as individuals with a subtype of anemia over all patients with anemia, not necessarily all patients included in the study.

EPO, erythropoietin; AUE, anemia of unknown etiology; IDA, iron deficiency anemia; Hb, hemoglobin; CKD, chronic kidney disease; HD, hemodialysis; N/A, not available.
Relationship between EPO levels and type of anemia

The majority of studies found obvious differences between EPO levels in IDA as compared with AUE, with levels often being significantly higher in individuals with IDA.4–6,11,16,17 In the study by Ferrucci et al.,18 EPO levels in AUE were lower than EPO levels measured in non-anemic controls and significantly lower than levels in individuals with IDA.

Five studies5–11,16,17,18 consistently showed no correlation between increased EPO levels and the severity of anemia in individuals with AUE. Conversely, these same studies also confirmed a strong inverse relationship between EPO levels and the degree of anemia in IDA.

The meta-analysis showed that EPO levels in AUE were significantly lower than in IDA (pooled RoM 0.7210; 95% CI 0.7052–0.7372; P-value < 0.00001; Fig. 2) and in ACD (pooled RoM 0.8995; 95% CI 0.8362–0.9677; P = 0.004; Fig. 3). Finally, EPO levels in AUE were slightly higher when compared to levels in anemia of CKD (pooled RoM 1.0940; 95% CI 1.0557, 1.1337; P < 0.00001; Fig. 4). In all analyses there was high statistical heterogeneity.

Discussion

The relationship between EPO and hemoglobin in elderly individuals with AUE has not been extensively studied and available evidence has not been comprehensively summarized. In the present study, we showed that EPO levels in AUE are lower when compared to other forms of anemia, particularly IDA and ACD, and only slightly higher than in patients with anemia of CKD. However, EPO levels remain generally higher than the levels measured in non-anemic patients. Additionally, there is a lack of correlation between the severity of anemia in AUE and the EPO level. In contrast, IDA displays a clear correlation, with EPO levels being highest in individuals with the...
lowest hemoglobin levels. Taken together, these results suggest the possibility of a relative EPO deficiency (perhaps as a result of an abnormal EPO response or because of a blunted erythroid progenitor response to EPO) as a potential mechanism for AUE.

A major limitation of this study is the heterogeneity of the studies included in the meta-analysis. The meta-analysis was conducted using a technique that allows one to account for the diversity of methods that each study used to measure serum EPO levels (i.e. differences in reagents, equipment and laboratory parameters). While the method has been validated as a means of analyzing outcome variables and does provide a summary of the role of EPO levels in AUE, it remains only an approximate estimate. The fact that certain statistical information necessary to calculate the pooled results had to be estimated by indirect statistical methods also may have resulted in less precise estimates. Additionally, the calculations used to determine the ratio of means do not account for the hemoglobin levels found in the various etiologies of anemia. However, to analyze the EPO levels in such a way would require a regression analysis using individual patient data which unfortunately are not available.

Although we believe that our search was comprehensive, we cannot exclude the possibility of negative unpublished studies having been missed. Additionally, since AUE is a diagnosis of exclusion a possibility of some degree of misclassification in individual studies cannot be completely ruled out.

Finally, the use of IDA as a standard for comparison is a potential limitation to the interpretation of the results, largely because there is evidence that iron can activate hypoxia-inducible factor (HIF), resulting in a decrease in hepcidin levels and a subsequent increase in EPO levels. Data regarding the use of iron supplementation prior to study involvement is not available, and it is possible that the elevation in EPO levels seen in this cohort might partially be attributable to the use of iron supplementation.

The pathogenesis of AUE remains speculative and it remains entirely possible that there are other factors contributing to the mechanism underlying AUE. In some studies pro-inflammatory cytokines (specifically, interleukin-6) were not found to be significantly elevated in AUE. It has also been suggested that aging is associated with an elevated level of pro-inflammatory cytokines leading to elevated levels of hepcidin (a negative regulator of iron absorption across intestinal mucosa). However, a study by Lee et al confirmed that hepcidin levels were not elevated in individuals suspected of having AUE, again supporting the notion that chronic inflammation is unlikely to be central to the pathogenesis of AUE. Other studies have suggested alternative mechanisms. The relative EPO deficiency detected across several studies may represent an early manifestation of renal disease 11. However, it remains unlikely that mild renal dysfunction is likely to be the sole mechanism of anemia. The studies included in the meta-analysis required the eGFR to be less than 30 mL/min/1.73 m² in order to be classified as anemia of chronic kidney disease although many of these patients excluded from this category may have had evidence of mild-to-moderate renal impairment (e.g. creatinine clearance between 30 and 60 mL/min). One study has addressed the threshold at which diminished creatinine clearance would result in anemia. Although the study suggested a trend toward an increase in the prevalence of anemia (significant only in the unadjusted analysis), only those individuals with an eGFR less than 30 mL/min/1.73 m² had a significantly high prevalence of anemia. Regardless, the possibility of misclassification cannot be completely ruled out since many studies used estimations of the eGFR such as the Cockroft–Gault formula, which are known to be more prone to biased results, and more accurate techniques exist. It is possible that patients included in the studies did in fact have renal impairment although categorized differently and the results must be interpreted while keeping this potential bias in mind. One final hypothesis that has been suggested is age-related defects in the hypoxia or EPO-sensing mechanism. Studies in mice have...
previously confirmed an inverse relationship between EPO response and oxygen transport capacity of the blood. The pathway involved in increased EPO secretion as a result of hypoxic conditions is complex. Hypoxia-inducible factor (HIF) is degraded by active prolyl hydroxylase inhibitors (PHIs) – a hypoxic state results in inhibition of PHIs, which leads to expression of HIF and subsequent upregulation of EPO. Although any state which contributes to hypoxia (including any type of anemia) should result in activation of the HIF pathway, a defect of the oxygen-sensing mechanism could increase the threshold for which serum EPO levels would produce an appropriate erythropoiesis response. This can certainly explain why an elevated level of EPO in the population of elderly with AUE fails to fully correct the anemia and why certain forms of anemia (particularly, IDA) display elevated levels of EPO in the absence of any dysfunction. However, the exact nature causing the dysfunction of these sensing mechanisms remains unclear and would warrant further study.

Finally, if the underlying mechanism is a relative deficiency of EPO, then the question of potential pharmacological interventions is raised. Erythropoietin-stimulating agents (ESAs) are used to treat anemic individuals with low EPO levels, specifically those whose anemia is secondary to malignancy or renal failure. It is entirely possible that ESA administration in AUE might be beneficial; however, this remains purely speculative and conclusions regarding the potential benefits of ESA administration for management of AUE cannot be commented on based on the results of our study, but it may warrant further investigation with regard to the benefits and risks of managing AUE more aggressively, in particular taking into account the potential for cardiovascular and thromboembolic events associated with ESA administration.

In conclusion, our findings suggest that EPO levels are generally elevated in elderly individuals with AUE, but remain inappropriately low, particularly when compared to IDA or ACD. This suggests that EPO may play a central role in the pathophysiology of this condition as a result of either a relative EPO deficiency, an abnormal EPO response to anemia or an abnormal erythroid cell response to EPO, and this state of ‘erythropoietin deficiency’ could constitute a distinct clinical entity. Further research is required to elucidate the mechanisms involved, and the potential value of ESAs.

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A.L.-L. conceived the study, A.L.-L. and S.S. designed the study, collected data and conducted the statistical analysis. A.X. contributed to the analysis. S.S. drafted the initial manuscript. All authors have reviewed the manuscript in its entirety.

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