BMC
Clinical Pathology

RESEARCH ARTICLE   **Open Access**

Accumulation of advanced glycation end (AGEs) products in intensive care patients: an observational, prospective study

Wendela L Greven*[1,2], Jitty M Smit[2], Johannes H Rommes[1] and Peter E Spronk[1,3,4]

Abstract

Background: Oxidative stress plays an important role in the course and eventual outcome in a majority of patients admitted to the intensive care unit (ICU). Markers to estimate oxidative stress are not readily available in a clinical setting. AGEs accumulation has been merely described in chronic conditions, but can also occur acutely due to oxidative stress. Since AGEs have emerged to be stable end products, these can be a marker of oxidative stress. Skin autofluorescence (AF) is a validated marker of tissue content of AGEs. We hypothesized that AGEs accumulate acutely in ICU patients.

Methods: We performed an observational prospective study in a medical surgical ICU in a university affiliated teaching hospital. All consecutively admitted ICU patients in a 2 month period were included. Skin AF was measured using an AGE reader in 35 consecutive ICU patients > 18 yrs. As a comparison, historical data of a control group (n = 231) were used. These were also used to calculate age-adjusted AF-levels (AF$_{adj}$). Values are expressed as median and interquartile range [P$_{25}$-P$_{75}$]. Differences between groups were tested by non parametric tests. P < 0.05 was considered statistically significant.

Results: AF$_{adj}$ values were higher in ICU patients (0.33 [0.00 - 0.68]) than in controls (-0.07 [-0.29 - 0.24]; P < 0.001). No differences in skin AF$_{adj}$ were observed between acute or planned admissions, or presence of sepsis, nor was skin AF$_{adj}$ related to severity of disease as estimated by APACHE-II score, length of ICU, hospital stay or mortality.

Conclusion: Acute AGE accumulation in ICU patients was shown in this study, although group size was small. This can possibly reflect oxidative stress in ICU patients. Further studies should reveal whether AGE-accumulation will be a useful parameter in ICU patients and whether skin AF has a predictive value for outcome, which was not shown in this small study.

Background

Patients admitted to the intensive care unit (ICU) may develop multiple organ dysfunction syndrome (MODS). The pathogenesis of this deterioration of organ function involves oxidative stress caused by reactive oxygen species (ROS) [1]. Indeed, the prognosis and outcome of patients admitted to the ICU is dependent on the degree of oxidative stress [2]. Oxidative stress is associated with most conditions requiring intensive care: but how could oxidative stress be measured? Interestingly, no common

strategy exists about its measurement and many methods of estimating oxidative stress have proven unreliable [3]. Moreover most parameters need complicated blood sample measurements.

Advanced glycation endproducts (AGEs) accumulate with oxidative stress and can be very easily and quickly measured, non-invasively. AGEs comprise a diverse class of compounds that link to proteins and accumulate in long-lived tissues. Several AGEs encompass a characteristic autofluorescence pattern when illuminating skin [4]. Using this specific property; skin AGE accumulation can be assessed by the AGE reader, a non-invasive, quick method, that has been thoroughly validated with AGEs measured in skin biopsies [4]. AGEs can be formed by

* Correspondence: wendelagreven@hotmail.com

[1] Department of Intensive Care Medicine, Gelre hospitals, location Lukas, Apeldoorn, the Netherlands
Full list of author information is available at the end of the article

several pathways [5]. In addition to the classical pathway, known as the Maillard reaction, other pathways are important, such as formation by reactive carbonyl compounds, which may form rapidly under oxidative stress [6]. In contrast to most other markers of oxidative stress, AGEs, once formed are stable compounds. They accumulate with age and play an important role in the development of end organ damage in several conditions such as diabetes, atherosclerosis and renal failure [7-9]. Two mechanisms may play a role. First, AGE modification directly alters the function and structure of extracellular matrix proteins [10]. Second, AGEs modulate cellular functions through ligation of specific cell surface receptors, such as the receptor for AGEs (RAGE) [11].

AGE accumulation has been described merely in chronic diseases. But since oxidative stress also augments AGE formation, and oxidative stress occurs in patients in the ICU, we hypothesize an acute rise of AGE levels in patients admitted to the ICU.

Methods
Design
This observational study, without interference with usual care was carried out in a university affiliated teaching hospital with an ICU with a mixed medical-surgical closed-format setting with 10 beds. Due to the observational nature of the study without changes in therapy, the requirement for obtaining informed consent was waived by the institutional review board.

AGE measurements
AGE levels were measured with the AGE-reader, a non-invasive, portable device, which has been thoroughly validated with AGEs measured in skin biopsies [4], DiagnOptics BV, Groningen, the Netherlands. All measurements were performed within 24 hours after admission, consistently by the same observer (WLG). The AGE-reader illuminates a skin surface of 1 cm^2, with an excitation light source between 300-420 nm. Only light from the skin is measured with a spectrometer in the 300-600 nm range, using 200 um glass fiber. Autofluorescence (AF) will be calculated as follows: ratio between average light intensity, measured in the range 420 - 600 nm and the average light intensity in the range 300-420 nm (emission/excitation) and this ratio is expressed as arbitrary units. Three consecutive measurements, from each patient were taken of which the mean was calculated.

Patients
Patients were included if skin AF could be measured within 24 hrs after admission. Patients with coloured skin were excluded, because the AGE reader had not yet been validated in patients with coloured skin. Patients with diabetes, liver cirrhosis or renal clearance below 35 ml/min were also excluded, because these conditions are known to cause AGE accumulation.

Controls
A formerly studied group of healthy volunteers (231 individuals, seen in the outpatient clinic for preoperative evaluation without a history of diabetes, cardiovascular events or renal disease, and all within ASA Class I or II criteria) was used to verify normal AGE levels in healthy individuals (see reference [12] for details)[12]. AGE data were plotted against calendar age to yield a standard skin AF level for each age since AGEs accumulate with age (figure 1). Subsequently, the age-adjusted skin AF (AF_{adj}) was calculated according the following formula: skin AF_{adj} level = skin AF measured - (0.9378 + (0.0233 * age)) for both patients and controls. After the calculation the skin AF_{adj} level for controls approaches zero.

Statistics
Statistical analyses were performed using SPSS 9.5 for WINDOWS. Non parametric tests were applied. All values are expressed as (median [interquartile range; P_{25} - P_{75}]). A P-value < 0.05 was defined to indicate statistical significance.
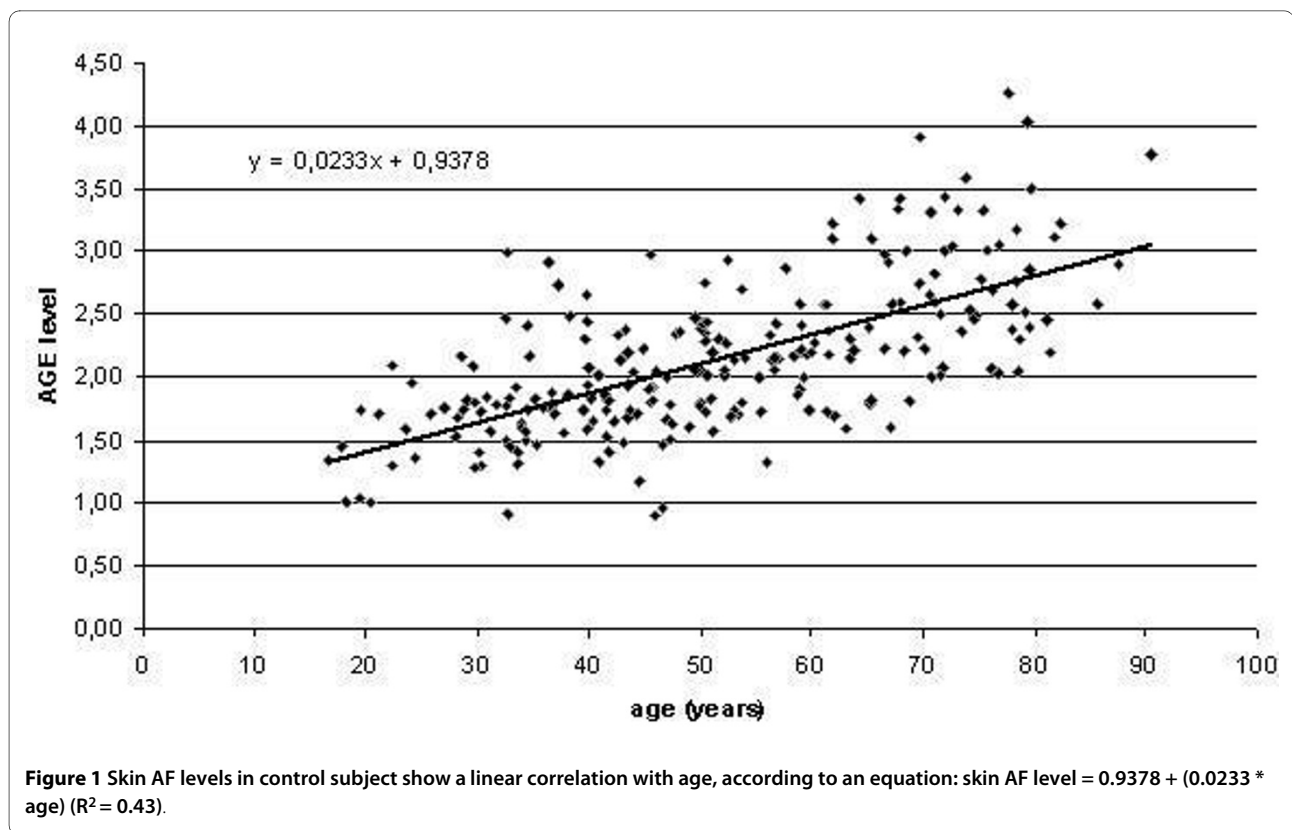
Results
Patients
Thirty-five consecutive ICU patients, 21 male, 14 female were included. The median age in patients was 74 years [57 - 80]. Patient characteristics are shown in table 1. APACHE-II scores as well as length of stay in the ICU were comparable to a one year period preceding this study (data not shown).

Skin autofluorescence measurements
Absolute skin AF levels were higher in ICU patients (2.81 [2.28-3.34]) as compared to controls (2.07 [1.74-2.47]; P < 0.001). However, ICU patients were older (74 [57-80] years]) than control subjects (51 [39-67] years]; P < 0.001). In view of the established relation between AGE-levels and calendar age, all further analyses were based on skin AF_{adj} (adjusted for age). After the correction for age, skin AF_{adj} was still higher in patients admitted to the ICU (0.33 [0.00 - 0.68]) than in controls (-0.07 [-0.29 - 0.24]; P < 0.001) (figure 2).

Associations with other parameters
No associations were found between skin AF-adj and disease severity as estimated by APACHE-II scores (R = 0.068), ICU length of stay (R = 0.004|), mortality (p = 0.905), nor with sepsis during ICU stay (p = 0.18), the reason for ICU admission (p = 0.544) or the urgency of admission (p = 0.292). In addition, no associations could be found between skin AF-adj and levels of albumin (R =

Figure 1 Skin AF levels in control subject show a linear correlation with age, according to an equation: skin AF level = 0.9378 + (0.0233 * age) (R^2 = 0.43).

0.036), highest glucose level (R = 0.016) or the lowest pH (R = 0.00) in the first 24 hours of ICU stay.

Discussion

This study shows acute AGE accumulation in ICU patients in a direct way by skin auto fluorescence. In this study the accumulation was not associated with disease severity, ICU length of stay or mortality.

Acute AGE accumulation is most likely mediated by oxidative stress, as the classical Maillard reaction is a slow non-enzymatic reaction. Oxidative stress causes formation of reactive carbonyl compounds, which react with protein to form AGEs [6]. Also blocking nitric oxide activity causes the production of reactive oxygen species [13]. Moreover binding of AGEs to RAGE generates intracellular reactive oxygen species [14,15]. This results in diminished reduced glutathione, and ascorbic acid. Depletion of glutathione leads to reduced glyoxalase-1 recycling and decreased in situ activity. Glyoxalase-1, however, has an important role in reducing the cellular AGE load [16,17]. Hence, an acute event could result in acute oxidative stress in the ICU patient, concomitant AGE accumulation, which itself could cause more oxidative stress, thus potentially causing a vicious cycle with progressive multiple organ failure.

The question is whether AGE accumulation is harmful? At least when evaluating chronic accumulation, AGE lev-

els predict future microvascular and cardiovascular events in diabetic patients better than HbA1c [8,9,18]. Moreover skin AGE accumulation predicts (cardiovascular) mortality in hemodialysis patients and in patients with type 2 diabetes [7,19]. In addition, interventions aiming to modulate AGE accumulation have proved to alleviate end-organ damage in animal models [20]. This may support the idea about AGEs playing a causal and potentially predictive role in end organ damage. In contrast to these chronic accumulation of AGEs, recent research is focussed on the less known acute AGE accumulation. One may hypothesize that this acute AGE accumulation, as seen in our study, may be harmful because of intracellular ROS formation as well as depletion of antioxidant mechanisms [15,16]. In ICU patients, one study showed elevated levels of soluble AGE receptors (sRAGE) at admission in septic patients [21]. Furthermore, application of an extracellular decoy for RAGE ligands, improves survival in mice after induction of sepsis, suggesting that RAGE is a central player in perpetuating the innate immune response [22]. Also, AGEs content measured in pericardial fluids seemed to have a prognostic factor after cardiac surgery [23] and plasma levels of sRAGE was associated with increased severity of lung injury and increased mortality in patients with acute lung injury [24]. In addition, plasma sRAGE levels predicted length of stay in ICU in patients after lung transplanta-

Table 1: Patient characteristics

	ICU patients
N	35
Age (years)	74 [57 - 80]
Skin AF level	2.81 [2.28 - 3.34]
Skin AF-adj	0.33 [0.00 - 0.68]
ICU LOS (days)	3.00 [2.00 - 6.25]
hospital LOS (days)	13.50 [8.50 - 24.25]
APACHE II	13.0 [10.0 - 16.0]
% died, during ICU-stay	14.3%
% ventilated	94.3%
Admission type	
% acute medical	17.1%
% acute surgical	40.0%
% planned	42.9%
First 24 hrs of ICU stay	
highest glucose level (mmol/l)	8.5 [6.5-11.9]
lowest pH	7.41 [7.36-7.44]
lowest albumin (g/l)	22 [15-24]

LOS = length of stay (days)
Values are expressed as median and IQR [P_{25} - P_{75}] or percentages

tion [25]. This is in concordance with our data finding elevated AGE levels already at admission in comparison to control subjects. However, we were not able to find an association with disease severity, ICU length of stay, nor with mortality. Besides differences in case mix and mortality rate, this difference might be explained by the fact that plasma sRAGE levels are not reflecting actual AGE
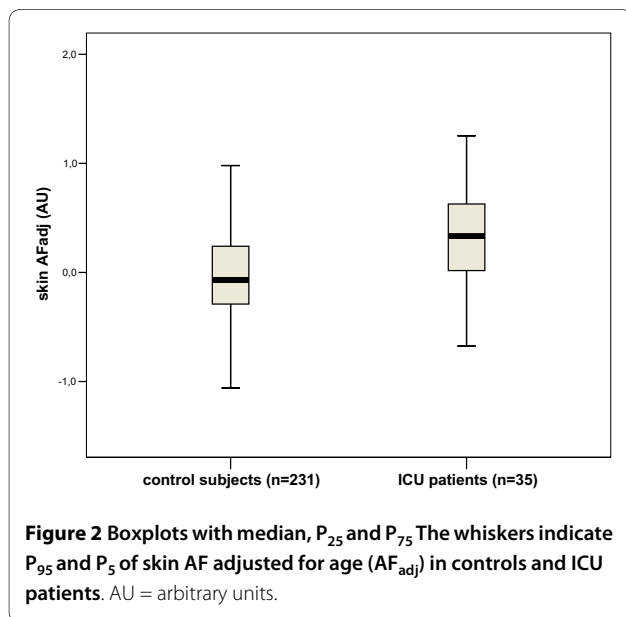
accumulation, thus yielding different results. We did not measure plasma sRAGE in our patients. More research, with larger numbers of patients, and in defined subgroups, should be carried out to address these issues.

Several study limitations need to be addressed. First, this is a small, single center study. Hence, the results may be quite different in other case-mixes in other ICUs. Secondly, this is a small series of patients. Nevertheless, they are probably a reasonable reflection of admissions to our ICU, because the composition of the patient characteristics did not differ with those in a 1 year period preceding this study. Thirdly, the AGE reader itself has not been formally validated for use in an ICU setting since all previous studies have been done in outclinic patients. One might argue that factors like edema could have influenced the results. Indeed, because water is not autofluorescent, values could be mildly reduced in the presence of substantial edema in the arm that was measured. However, this should be structurally addressed in further studies. Fourth; patients with conditions, known to cause AGE accumulation were not included in this study. So AGE accumulation is therefore most likely caused by the ICU admission itself. But the design of this study was cross-sectional, so skin AF values before ICU admission of these particular patients are unknown. Further studies, should maybe focus on longitudinal skin AF measurements to show acute accumulation with ICU admission in the same patient.

In conclusion, acute AGE accumulation occurs in ICU patients, which probably reflects oxidative stress. The group was too small to allow any conclusions on the possible predictive value of skin AF for prognosis for patients on the ICU. Further studies should reveal whether measurement of AGE-accumulation will be a useful parameter in ICU patients.

Conclusion

Acute AGE accumulation was shown in this small group of ICU patients, which probably reflects oxidative stress. The group was too small to allow any conclusions on the possible predictive value of skin AF_{adj} for outcome. Further studies should reveal whether AGE-accumulation will be a useful parameter in ICU patients.

Figure 2 Boxplots with median, P_{25} and P_{75} The whiskers indicate P_{95} and P_5 of skin AF adjusted for age (AF_{adj}) in controls and ICU patients. AU = arbitrary units.

Authors' contributions
WLG carried out the measurements, did the analysis and interpretation of the data, and wrote the manuscript
JMS has made substantial contribution to the design and revised the manuscript critically for important intellectual content
JHR has revised the manuscript critically for important intellectual content
PES has made substantial contribution to the design, the analysis of the results and revised the manuscript critically for important intellectual content
All authors read and approved the final manuscript.

Author Details
[1]Department of Intensive Care Medicine, Gelre hospitals, location Lukas, Apeldoorn, the Netherlands, [2]Department of Internal Medicine, Gelre hospitals, location Lukas, Apeldoorn, the Netherlands, [3]Department of Intensive Care Medicine, Academic Medical Center, Amsterdam, the Netherlands and [4]HERMES critical care group, Academic Medical Center, Amsterdam, the Netherlands

References
1. Abiles J, de la Cruz AP, Castano J, Rodriguez-Elvira M, Aguayo E, Moreno-Torres R, *et al.*: Oxidative stress is increased in critically ill patients according to antioxidant vitamins intake, independent of severity: a cohort study. *Crit Care* 2006, **10**:R146.
2. Motoyama T, Okamoto K, Kukita I, Hamaguchi M, Kinoshita Y, Ogawa H: Possible role of increased oxidant stress in multiple organ failure after systemic inflammatory response syndrome. *Crit Care Med* 2003, **31**:1048-1052.
3. Grune T, Berger MM: Markers of oxidative stress in ICU clinical settings: present and future. *Curr Opin Clin Nutr Metab Care* 2007, **10**:712-717.
4. Meerwaldt R, Links T, Graaff R, Thorpe SR, Baynes JW, Hartog J, *et al.*: Simple noninvasive measurement of skin autofluorescence. *Ann N Y Acad Sci* 2005, **1043**:290-298.
5. Baynes JW, Thorpe SR: Glycoxidation and lipoxidation in atherogenesis. *Free Radic Biol Med* 2000, **28**:1708-1716.
6. Miyata T, van Ypersele de SC, Kurokawa K, Baynes JW: Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. *Kidney Int* 1999, **55**:389-399.
7. Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC, *et al.*: Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005, **16**:3687-3693.
8. Meerwaldt R, Links TP, Graaff R, Hoogenberg K, Lefrandt JD, Baynes JW, *et al.*: Increased accumulation of skin advanced glycation end-products precedes and correlates with clinical manifestation of diabetic neuropathy. *Diabetologia* 2005, **48**:1637-1644.
9. Meerwaldt R, Lutgers HL, Links TP, Graaff R, Baynes JW, Gans RO, *et al.*: Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007, **30**:107-112.
10. Singh R, Barden A, Mori T, Beilin L: Advanced glycation end-products: a review. *Diabetologia* 2001, **44**:129-146.
11. Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, *et al.*: Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. *Proc Natl Acad Sci USA* 1994, **91**:8807-8811.
12. Lutgers HL, Graaff R, Links TP, Ubink-Veltmaat LJ, Bilo HJ, Gans RO, *et al.*: Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. *Diabetes Care* 2006, **29**:2654-2659.
13. Goldin A, Beckman JA, Schmidt AM, Creager MA: Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006, **114**:597-605.
14. Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM: Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J Biol Chem* 1997, **272**:17810-17814.
15. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL: Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab* 2001, **280**:E685-E694.
16. Bierhaus A, Chevion S, Chevion M, Hofmann M, Quehenberger P, Illmer T, *et al.*: Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. *Diabetes* 1997, **46**:1481-1490.
17. Thornalley PJ: Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Cell Mol Biol (Noisy -le-grand)* 1998, **44**:1013-1023.
18. Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, *et al.*: Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* 2005, **54**:3103-3111.
19. Lutgers HL, Gerrits EG, Graaff R, Links TP, Sluiter WJ, Gans RO, *et al.*: Skin autofluorescence provides additional information to the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in type 2 diabetes mellitus. *Diabetologia* 2009, **52**:789-797.
20. Alderson NL, Chachich ME, Youssef NN, Beattie RJ, Nachtigal M, Thorpe SR, *et al.*: The AGE inhibitor pyridoxamine inhibits lipemia and development of renal and vascular disease in Zucker obese rats. *Kidney Int* 2003, **63**:2123-2133.
21. Bopp C, Hofer S, Weitz J, Bierhaus A, Nawroth PP, Martin E, *et al.*: sRAGE is elevated in septic patients and associated with patients outcome. *J Surg Res* 2008, **147**:79-83.
22. Bopp C, Bierhaus A, Hofer S, Bouchon A, Nawroth PP, Martin E, *et al.*: Bench-to-bedside review: The inflammation-perpetuating pattern-recognition receptor RAGE as a therapeutic target in sepsis. *Crit Care* 2008, **12**:201.
23. Simm A, Wagner J, Gursinsky T, Nass N, Friedrich I, Schinzel R, *et al.*: Advanced glycation endproducts: a biomarker for age as an outcome predictor after cardiac surgery? *Exp Gerontol* 2007, **42**:668-675.
24. Calfee CS, Ware LB, Eisner MD, Parsons PE, Thompson BT, Wickersham N, *et al.*: Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. *Thorax* 2008, **63**:1083-1089.
25. Calfee CS, Budev MM, Matthay MA, Church G, Brady S, Uchida T, *et al.*: Plasma receptor for advanced glycation end-products predicts duration of ICU stay and mechanical ventilation in patients after lung transplantation. *J Heart Lung Transplant* 2007, **26**:675-680.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1472-6890/10/4/prepub