

The influence of cytomegalovirus and Epstein-Barr virus serostatus on haemoglobin levels and erythropoietin-stimulating agent responsiveness in patients on the transplant list with stage 5 chronic kidney disease

Abstract

Cytomegalovirus (CMV) seropositivity has been reported to be a major determinant of erythropoietin-stimulating agent (ESA) resistance in patients with chronic kidney disease (CKD). It is hypothesised that prior CMV infection induces significant changes in T-cell subtypes that promote bone marrow resistance to ESA. We examined whether CMV or Epstein-Barr virus (EBV) serostatus influenced haemoglobin (Hb) levels and/or ESA resistance in our population of CKD stage 5 patients.

Data on CMV and EBV serology, age, sex and Hb was collected on 1,417 patients presenting for a renal transplant from 2000 to 2009 in Ireland. Patients were split into four groups (CMVneg/EBVneg, CMVpos/EBVneg, CMVneg/EBVpos, and CMVpos/EBVpos) and analysis of variance was performed to examine whether CMV and EBV serostatus influenced Hb levels pre transplantation. Data was then collected on 117 patients currently on the transplant pool from Beaumont and Tallaght Hospitals. CMV and EBV serostatus, Hb, ESA dose and other parameters associated with ESA responsiveness (iron studies, B12/folate, albumin, PTH, diabetes, vascular access, dialysis adequacy) were collected. Multivariate analysis was performed to determine factors associated with increased ESA dosage.

CMV positivity was found to have no effect on Hb levels or ESA dosage. Likewise, EBV serostatus had no effect on these parameters. First, analysis of 1,417 patients showed no difference in mean Hb between the various CMV/EBV serostatus groups. Second, analysis of patients currently on the transplant pool also showed that there was no difference in mean Hb between CMV and EBV groups. Analysis of a subpopulation of haemodialysis patients alone showed that CMV positivity was associated with a higher mean Hb and a lower mean ESA dose.

Our results contrast with those of a recent report by Betjes *et al.* linking ESA resistance to CMV seropositivity. No association was found between CMV or EBV serostatus and Hb levels or ESA dosage. CMV seropositivity is not associated with increased ESA requirements in our population. These conflicting results may be due to differences in patient demographics and a lower target Hb level in our study.

Keywords: Haemoglobin, erythropoietin, CMV, EBV, renal disease.

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Introduction

Anaemia associated with chronic kidney disease (CKD) is a common problem that is associated with reduced quality of life and increased morbidity and mortality.¹ The main cause is erythropoietin (Epo) deficiency. Epo is secreted by the peritubular capillary cells of the kidney and its production decreases when glomerular filtration rate (GFR) falls below approximately 40ml/min. Other causes of anaemia in CKD are decreased red blood cell (RBC) survival, bone marrow dysfunction in the setting of uraemia, increased RBC loss from uraemic gastritis and blood loss associated with the dialysis procedure. The main treatment is administration of erythropoiesis-stimulating agents (ESAs) and ensuring adequate iron stores. ESAs are similar to the naturally occurring protein Epo, which stimulates RBC formation (erythropoiesis). Available ESAs include Epo, epoetin alfa (Procrit/Epogen), epoetin beta (NeoRecormon) and darbepoetin (Aranesp).^{2,3} However, patients differ in their sensitivity to ESA treatment, and in those that are hyporesponsive, a larger dose is required to maintain the haemoglobin (Hb) at an adequate level.⁴ There is controversy over the optimal Hb target, but it is usually taken to be 11-12g/dL. Of note, complete correction of anaemia to Hb levels higher than these values may be associated with increased cardiovascular risk.^{5,6}

There are a number of factors that cause Epo hyporesponsiveness, including iron deficiency, systemic inflammation, hyperparathyroidism, vitamin B12 or folate deficiency, and co-morbidities such as diabetes, ongoing blood loss and inadequate dialysis.⁷

There has been recent evidence that prior exposure to cytomegalovirus (CMV) infection resulting in seropositivity to CMV is associated with resistance to Epo treatment in CKD patients.⁸ CMV is a herpes DNA virus that causes infectious mononucleosis and is commonly acquired in the teenage years. Often, the infection is asymptomatic or associated with a mild flu syndrome. The prevalence of seropositivity increases with the age of the population, such that approximately 50% of 50 year olds are seropositive.⁹ It does not cause significant clinical problems unless a patient becomes immunosuppressed such as in renal transplant patients. CMV infection in immunocompromised individuals can be life threatening, such that reactivation of the virus can cause CMV retinitis and pneumonitis. Furthermore, it is associated with distinct changes in peripheral T-cell populations. Betjes *et al.* have shown that CKD patients with positive CMV serology required significantly more Epo to achieve target Hb values (median 12,000 vs. 6,300 units/week; p=0.02). They provide evidence that this Epo-resistant state is due to a CMV-mediated change in peripheral T-cell subsets. Prior CMV infection is associated with expanded CD4+ CD28 null T-cells that are pro-inflammatory and mediate bone marrow resistance to the effects of Epo.¹⁰

This study examined if there was an association between CMV serostatus and Epo responsiveness in our CKD population. A retrospective analysis of 1,417 CKD patients presenting for renal

Table 1: Erythropoietin dose conversion.

Darbepoetin alfa (Aranesp) mg/week	Epoetin beta (NeoRecormon) iu/week	Methoxy polyethylene glycol-epoetin beta (Mircera) mg/month
<40	<8000	120
40-80	8000-16000	200
>80	>16000	360

transplantation was conducted to determine whether CMV serostatus correlated with Hb values prior to transplantation. Thereafter, a detailed analysis of 117 patients on the transplant waiting list in Beaumont and Tallaght Hospitals was performed to determine factors associated with Epo resistance in a multivariate analysis. In addition, we included serostatus for Epstein-Barr virus (EBV) infection in these patients as we hypothesise that infection with EBV may have a similar effect on Epo requirement. EBV is also a herpes virus like CMV and any findings in this area would be novel.

Methods

Hospital data for the 1,417 patients who were transplanted in Ireland over the past 10 years (2000-2009) were collected. This information was obtained from hospitals across Ireland. No confidential patient details such as names were recorded and therefore ethics approval was not required before data collection. The data was then used in a retrospective analysis to determine whether CMV and EBV seropositivity affected Hb levels. The patients in our list were divided into four subgroups according to their serostatus, namely CMV+EBV+, CMV+EBV-, CMV-EBV+, and CMV-EBV-. Analysis of variance (ANOVA) was used to examine the mean Hb level for the various EBV and CMV status groups, and so a comparison was made to determine if the presence of CMV or EBV infection had an effect on Hb levels. The significance of other confounding factors such as age at transplantation, sex of patient and the year of transplant were also determined for these patients. Next, a sub-analysis of the 117 patients on the renal transplant list from Beaumont and Tallaght Hospitals was conducted. The criteria for selection were for the patient to have been on the transplant list from January 2010 and to be a patient at either of these hospitals. These patients in the transplant pool were selected for study, as a patient's CMV and EBV serostatus are now routinely determined prior to being put on the transplant list. Patient information was collected using case notes, referral letters and hospital databases (Clinical Vision and PIPE for Beaumont Hospital renal patients). The data collected was then categorised and entered into an electronic database.

First, the mean Hb levels for CMV+, CMV-, EBV+ and EBV- patient groups were calculated and differences among these values were compared. Second, the different types of Epo used by these

Table 2: Standardised scoring system for erythropoietin dosage.

Erythropoietin score	Aranesp	NeoRecormon	Mircera
0	0	0	0
1	10	2000	30
2	20	4000	60
3	30	6000	90
4	40	8000	120
5	50	10000	180
6	60	12000	240
7	70	14000	-
8	80	16000	360
9	90	18000	-
10	100	20000	480
11	110	22000	-
12	120	24000	-
13	130	26000	-
14	140	28000	-
15	150	30000	-

patients and the popularity of each were examined. Analysis of Epo dose required the use of the conversion system from either ESA (Table 1). Darbepoetin alfa (Aranesp) and epoetin beta (NeoRecormon) doses were quoted weekly, while Mircera was given monthly. A standardised scoring system was derived to allow comparison of differences in dosage of the various Epo types (Table 2). The validity of results derived from using this system was verified by standardising Aranesp dose from micrograms into units in the same manner as Betjes *et al.*⁷ This was done by multiplying the Aranesp dose by 200. Third, a multivariate analysis was performed using the patient data and these standardised doses to investigate the effect CMV

and EBV serostatus has on Epo dose and Hb concentration, while simultaneously looking at the effects of other key variables known to cause Epo hyporesponsiveness. The specific variables included were serum iron, vitamin B12 and folate levels, serum ferritin, transferrin saturation, serum albumin, diabetic status, parathyroid hormone (PTH) levels and the use of an angiotensin II receptor blocker (ARB) or ACE inhibitors (ACEIs).⁷ This enabled a comparison of the extent of influence each variable had on Epo dose and Hb levels.

Results

The retrospective analysis showed that there were no significant differences in Hb levels among the various groups. Overall, the CMV-EBV- group reported high mean Hb levels, while the CMV+EBV- had the lowest mean Hb (Table 3). This difference in Hb levels between the seropositive and the seronegative patients was not significant when compared to the other confounders. However, the variables that were found to have significant effects on Hb levels were the gender of the patient and the year of the transplant (Table 4).

The clinical and demographic data for the 117 patients on the transplant pool are outlined in Table 5. Of the 117 patients, 43 were female and 74 were male. Mean Hb was slightly higher in the CMV+ group (11.36g/dL) compared to the CMV- group (11.30g/dL). Aranesp proved to be the most popular Epo used by the patients.

In the transplant pool, there were more people with diabetes in the CMV+ group (16.7% compared to 7.8%). In addition, the patients in this study were younger than those in Betjes' study (mean age 47.3 years in the CMV+ group and 49.8 in the CMV- group, compared to 57.4 years in Betjes' haemodialysis patients). Albumin levels were roughly the same in both groups (CMV- 40.16g/dL; CMV+ 40.09g/dL). Serum iron was higher in the CMV- group (15µg/dL), and ferritin was higher in the CMV+ group (434ng/ml). The mean Epo score (Table 2) for the CMV+ group was 0.44 higher than the CMV- group.

Of note, analysis of these patients also showed no difference in Hb levels among the various serostatus groups. The EBV patients had the highest mean Hb level of 11.57g/dL, but it was not statistically significant (Figure 1).

Table 3: Analysis of variance (ANOVA) examining equality of haemoglobin for EBV and CMV serostatus groups.

EBV-CMV status	Mean haemoglobin (g/dl)	Standard deviation for haemoglobin (g/dl)	Minimum haemoglobin (g/dl)	Maximum haemoglobin (g/dl)
Neg-Neg	12.3	1.8	7.1	17.8
Neg-Pos	11.8	1.4	8.8	14.1
Pos-Neg	12.3	1.6	7.2	17.8
Pos-Pos	12.3	1.6	7.6	16.3

Table 4: Significance of confounders of age at transplantation, sex of patient and year of transplant.

Variable	Partial sum of squares	Degrees of freedom	Mean sum of squares	F	P value
EBV-CMV	6.15697596	3	2.05232532	0.81	0.4888
Age at transplant	195.661828	69	2.83567866	1.12	0.2423
Sex	20.4179786	1	20.4179786	8.05	0.0046
Year	112.486054	10	11.248604	4.44	<0.0001

Table 5: The clinical and demographic characteristics of patients on the 2010 transplant waiting list.

Variable	CMV positive (n=54)	CMV negative (n=63)	P value
% male gender	32	42	0.441
Age (mean)	47.3	49.8	0.263
Diabetes (%)	16.7	7.8	0.387
Dialysis type:			
% Haemodialysis	0.54	0.52	0.623
% Peritoneal dialysis	0.32	0.43	
% Pre-dialysis/not on dialysis	0.14	0.05	
Erythropoietin type			
% Aranesp	0.57	0.49	0.557
% NeoRecormon	0.30	0.23	
% Mircera	0.04	0.06	
None	0.09	0.22	
Erythropoietin score (median [IQR])	3 [1.5-4]	3 [2-5]	0.505
Albumin (g/dL) (mean)	40.09	40.16	0.689
Hb (g/dL) (mean)	11.36	11.30	0.780
B12 (µg) (median [IQR])	353 [249-457]	327.5 [258.5-469]	0.965
Folate (µg) (median [IQR])	11.65 [8.4-15.1]	12.7 [8.7-16.5]	0.588
Serum Fe (µg/dL) (median [IQR])	12 [9.3-15]	13.6 [9.9-18.25]	0.089
Ferritin (ng/mL) (median [IQR])	405 [253-621.5]	400 [213-571.5]	0.507
Transferrin saturation (median [IQR])	30.12 [24.55-37.14]	31.03 [24-41.985]	0.432
PTH (pg/ml) (Median [IQR])	321 [215-545]	340.5 [171.5-598]	0.316
Weight (kg) (median [IQR])	75.75 [63-86.8]	72.4 [63.4-84.1]	0.649
Urea reduction ratio (%) (median [IQR])	73 [66-76.4]	69 [67-75]	0.333
% EBV-positive	0.96	0.87	0.172
% on ACEI/ARB	0.39	0.40	0.026

Table 6: The effect of CMV serostatus on haemoglobin levels for patients on haemodialysis.

CMV status	Mean haemoglobin (g/dl)	Standard deviation for haemoglobin (g/dl)	Median haemoglobin (g/dl)
Neg.	10.55455	1.025637	10.9
Pos.	11.44483	1.130609	10.9

Table 7: Comparison of mean haemoglobin levels (g/dL) for patients on erythropoietin therapy and those without therapy.

Treatment status	CMV positive	CMV negative
Epo treatment	11.1462	10.7714
No Epo	12.5	12.3786

Table 8: The significance of the various factors on erythropoietin dose.

Variable	Partial sum of squares	Degrees of freedom	Mean sum of squares	F	P value
CMV status	0.287095976	1	0.287095976	0.70	0.4065
High PTH	1.06657753	1	1.06657753	2.60	0.1128
High folate	0.02646765	1	0.02646765	0.06	0.8004
Low serum Fe	0.457643219	1	0.457643219	1.12	0.2956
High ferritin	1.77227242	1	1.77227242	4.32	0.0426
High transferrin saturation	0.152055625	1	0.152055625	0.37	0.5452
ACEI/ARB	0.338380885	1	0.338380885	0.83	0.3678
Diabetes	0.000193397	1	0.000193397	0.00	0.9828

Separate analysis conducted for patients on haemodialysis (excluding patients on peritoneal dialysis) showed that the mean Hb was higher in the CMV+ group (11.44g/dL) than in the CMV- group (10.55g/dL, $p=0.016$) (Table 6). Mean Hb for patients not on Epo was higher than for those not on treatment (CMV+ patients: 12.50g/dL vs. 11.15g/dL; CMV- patients: 12.38g/dL vs. 10.77). CMV+ patients in both categories had higher Hb levels than CMV- patients (Table 7).

Patients not on Epo therapy and those receiving Mircera treatment were excluded, as they were few in number and would lower accuracy. The Epo doses of the remaining patients were then standardised by Betjes' method and this data was represented on a box plot (Figure 2).¹⁰ This showed that the CMV- patients required a higher median Epo dose than CMV+ patients, 8000U/wk and 6000U/wk, respectively. Univariate analysis showed that ferritin was the only variable that had a significant effect on Epo dose ($p=0.0253$), while the multivariate analysis showed that serum iron had a significant effect on erythropoietin dose ($p=0.0441$) (Table 8).

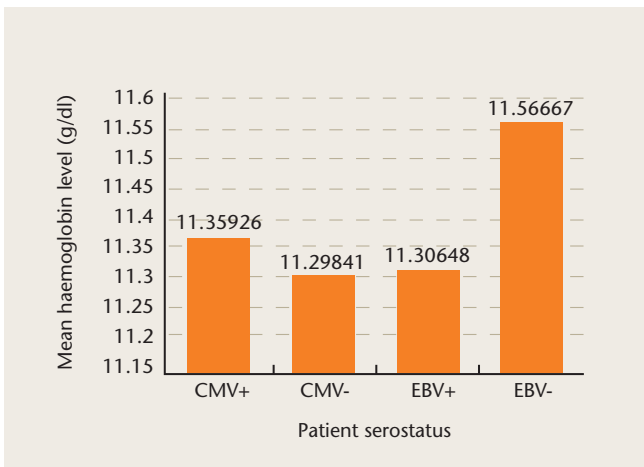


FIGURE 1: Relationship between patient serostatus and mean haemoglobin levels showed no difference in haemoglobin levels among the various serostatus groups.

Discussion

This study examined 1,417 CKD patients transplanted in Ireland over the past ten years and found that neither their CMV nor EBV serostatus affected their mean Hb levels at the time of transplantation. The sex of the patient and the year of transplantation had an effect on Hb levels. It is well known that women have lower Hb levels, and any effect the year of transplantation might have may in fact represent changes in clinical practice, such as different target Hb in current practice. The lack of an effect of CMV serostatus on Hb is similar to the findings by Betjes *et al.*¹⁰ However, any potential effect of CMV serostatus on Hb values may be masked by the fact that most CKD patients are receiving ESA agents and clinical management involves increasing the dose of ESA to achieve Hb levels within the target range. Betjes *et al.* concluded that CMV seropositivity affects anaemia management in CKD by significantly decreasing the bone marrow responsiveness to ESA, and showed that patients who are CMV+ require significantly higher doses of ESA agents.¹⁰ Moreover, Betjes *et al.* examined a subpopulation of patients not on ESA therapy and found that CMV+ patients were on average

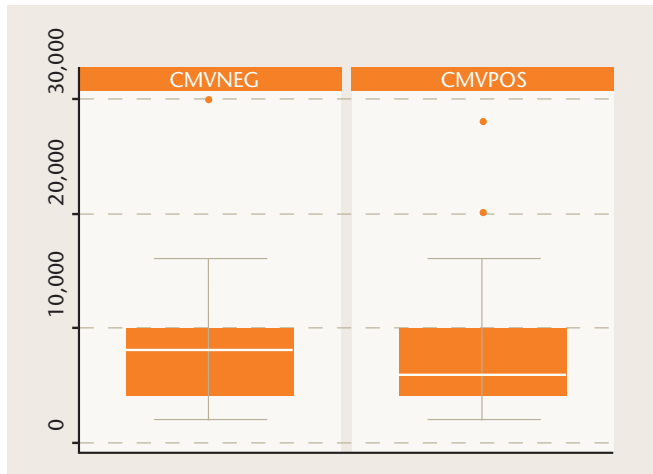


FIGURE 2: Box-plot of erythropoietin doses standardised by Betjes' method. CMV- patients required a higher median erythropoietin dose of 8000U/wk in comparison to the CMV+ patients, who required 6000U/wk.

more anaemic. In this study, CMV serostatus did not affect responsiveness to ESA agents in the CKD population and these results were not in agreement with Betjes *et al.*'s findings. Analysis of patients on haemodialysis shows that the mean Hb is higher in CMV seropositive patients (11.44g/dl) than in the CMV seronegative group (10.55g/dl). However, CMV+ patients received a lower mean dose of ESA. This is a novel finding, as previous studies found that CMV seropositivity leads to higher ESA requirements.¹⁰ In the multivariate analysis, the only factor positively associated with ESA dose was serum iron, and it is well known that iron deficiency causes ESA hyporesponsiveness. The marked difference in the results here may possibly be due to differences in patient demographics between study populations. Moreover, the mean Hb in our patient population was lower than that in Betjes *et al.*'s study. Overall, our patient population received a lower mean dose of ESA (mean

6871.9U/wk for haemodialysis patients) compared to those in Betjes *et al.*'s study (10,399U/wk). This may be because the target Hb level at our institutions is lower, or perhaps the effect of CMV serostatus on ESA responsiveness only manifests when attempting to achieve a higher Hb level in these patients. The mean age of our population was younger than those studied by Betjes *et al.* (47.3 years vs. 57.4 years). This may be an important factor because the effect of CMV on T-cell subpopulations (enhanced CD4+, CD28- population) is age dependent. We also had a lower percentage of patients with diabetes in our study, and there was a difference in the percentage of patients with diabetes between groups. This may be important because of the influence of diabetes on anaemia. Of note, renal impairment due to microvascular complications of diabetes could lead to impaired Epo production and, ultimately, anaemia.¹¹ Additionally, the patients on the transplant list were perhaps in better health by virtue of being younger than those with end-stage renal disease included in Betjes *et al.*'s study.

The inconsistency between the results presented by Betjes *et al.* and those in this study demonstrates that the relationship between CMV serostatus and Epo dose is still unclear. Further large-scale studies should be conducted to definitively determine the association between patient CMV serostatus and required Epo dose, if any. Currently, patients with renal disease may pay thousands annually for Epo treatment, and costs can be as much as double that paid for the alternative treatment of RBC transfusions.¹² Careful control and surveillance of influencing factors, such as glucose control in patients with diabetes and prevention of iron deficiency, may help to lower Epo requirements, and thereby decrease the cost for patients.

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