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Postoperative estradiol levels associate with the development of primary graft dysfunction in lung transplant patients

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Abstract

Primary graft dysfunction (PGD) frequently complicates lung transplantation in the immediate postoperative period. Both female gender and estradiol modulate the body's response to injury and may influence the rate of alveolar fluid clearance. We hypothesized that female gender and higher estradiol levels would be associated with a lower risk of PGD after lung transplantation. We measured plasma estradiol levels pre-operatively, 6 hours postoperatively and 24 hours postoperatively in a cohort of 111 lung transplant recipients at two institutions. The mean age was 57 years (+/- 12.5) and 52% were female. The median postoperative estradiol level was 63.9 pg/ml (IQR 28.8–154.3) in men and 65.1 pg/ml (IQR 28.4–217.2) in females. Contrary to our hypothesis, higher estradiol levels at 24 hours were associated with an increased risk of PGD at 72 hours in males (p=0.001). This association was preserved when accounting for other factors known to be associated with PGD. However, there was no relationship between gender and risk of PGD or between estradiol levels and PGD in females. These findings suggest that there may be different biologic effects of estrogens in males and females and highlight the importance of considering gender differences in future studies of PGD.

Keywords

Lung transplantation; gender; acute lung injury; acute respiratory distress syndrome; estrogen; alveolar fluid clearance

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Introduction

Lung transplantation is a therapeutic option for patients with chronic advanced lung diseases. Despite improvements in recent years, many patients still develop acute lung injury in the days immediately following transplantation, termed primary graft dysfunction (PGD). (1) PGD not only causes short term morbidity but also increases the risk of developing bronchiolitis obliterans due to chronic rejection in the years following transplantation. (2, 3) There are several donor, recipient and operative factors that are associated with an increased risk of PGD. Donor factors including age, race, female gender, smoking history, and trauma increase the risk of PGD, as do recipient pulmonary hypertension, diffuse parenchymal lung disease, prolonged organ ischemic time, and the use of cardiopulmonary bypass. (4–7)

Both gender and sex hormones are important in modulating the body's response to injury or infection. Plasma estradiol levels are elevated in both men and women in a variety of critical illness settings including severe infection(8) and traumatic injury; (9, 10) elevated levels are associated with mortality in these settings. Although a subset of patients with sepsis and trauma develop acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS), the effect of gender on risk and outcomes from ALI is uncertain. In patients with ALI, female gender has been associated with a lower risk of death in both a retrospective study in India (11) and a large epidemiologic study (12) although the mechanism of protection was not examined in these studies.

The association of gender and sex hormones with the risk of PGD after lung transplantation is unknown. We have recently reported that women with ALI demonstrated more rapid rates of alveolar fluid clearance than men. (13) However, plasma estradiol levels were not measured in that study. Given the uncertainty of the relationship between gender, estradiol and outcomes from ALI and PGD, we conducted a prospective study of the role of estradiol levels in the development of PGD following lung transplant. We utilized a cohort of male and female patients who underwent lung transplantation to test the hypothesis that female lung transplant recipients would have higher postoperative estradiol levels and that both female gender and higher estradiol levels would be associated with a lower risk of developing primary graft dysfunction.

Methods

Patients

This study is a substudy of the Lung Transplant Outcomes Group (LTOG) cohort study. (5, 14–19) For the current study, 111 consecutive lung transplant recipients were enrolled at 2 LTOG centers between October 2006 and May 2008 (Columbia University and the University of Pennsylvania) and followed prospectively for the development of primary graft dysfunction after lung transplantation. Citrated plasma was collected at three time points: pre-transplantation, 6 hours post transplantation, and 24 hours post transplantation as described previously. (20) Not all patients were available for sample collection at all 3 time points. After collection, plasma samples were aliquoted and stored at –80C. Clinical data were collected pre-transplantation and for 72 hours post-transplantation. The primary outcome was the development of severe (Grade 3) PGD within the first 72 hours following transplant. PGD grading was performed according to ISHLT criteria at T0, 24, 48, and 72 hours following transplant. (4, 21) Chest radiographs were independently read by two separate investigators; adjudication was performed when interpretation differed. The agreement between readers was good (kappa for grade resultant from individual reads=0.95). Grade 3 PGD was defined as the presence of infiltrates in the lung allograft(s) on chest radiograph and severe hypoxemia with a PaO₂/FiO₂ ratio <200 as previously

described. (15, 20–22) The clinical cohort study was approved by the institutional review boards at both enrolling institutions. All patients provided written informed consent.

Plasma Estradiol Measurements

Estradiol was measured in duplicate in EDTA plasma using a commercially available ELISA kit (BioCheck, Inc., Foster City, CA). The mean intra-assay CV was 10.9% (range 5.3–18.4) and the mean inter-assay CV was 22.7% (range 1.8–58.4).

Statistical Analysis

Estradiol levels were non-normally distributed (by Shapiro-Wilk test, $p < 0.001$) and are expressed as median (IQR). Differences in estradiol levels between groups were compared using non-parametric tests (Mann Whitney U test for comparison of 2 groups and Kruskal-Wallis for comparison of more than two groups). Generalized estimating equations (GEE) were used to identify differences in estradiol levels over time and across study subjects. Chi square or Fisher's exact test were used to compare proportions. We *a priori* planned stratified analyses according to gender. A multivariable logistic regression analysis was done using the presence of grade 3 PGD at any grading time point within the first 72 hours as the dependent variable and natural log transformed estradiol as the independent variable. Normality after log transformation was confirmed with a Q-Q plot. Potential confounders were added to the logistic regression model one at a time and an odds ratio was computed. A change in odds ratio (OR), after inclusion of a covariate, of greater than 20% was used to identify confounding. For all analyses, $p < 0.05$ was considered statistically significant. Analysis was done using SPSS version 19.0.0 for Macintosh (IBM Corporation, Somers, NY) and STATA 11.1 software (STATA Corp., College Station, TX).

Results

Donor and recipient clinical characteristics and rates of primary graft dysfunction

The characteristics of the recipients and donors are shown in Table 1. PGD was defined as the development of grade 3 PGD at any time during the first 72 hours following lung transplantation. For both patients with PGD and those without PGD, the female recipients were more likely to have a female donor than male recipients (71% female donors for female recipients, 30% female donors for male recipients, $p < 0.001$). However, there were no differences in 24-hour postoperative estradiol levels in any of the donor-recipient sex combinations (ie. Female recipients of female lungs versus male recipients of female lungs). The recipient age, race, diagnosis leading to transplantation, type of transplant (single vs. double), and use of cardiopulmonary bypass (CPB) were similar between male and female recipients. In both genders, recipients with PGD were less likely to have a diagnosis of cystic fibrosis and more likely to have a diagnosis of pulmonary arterial hypertension. Also, more patients with PGD were on cardiopulmonary bypass during the operation. There was no difference between recipient males and females with respect to development of grade 3 PGD at any point in the first 72 hours postoperatively (22.6% males vs 32.8% females, $p = 0.291$) or presence of grade 3 PGD on any postoperative day (Table 2). Donor gender was associated with PGD graded on day 3: 54.2% of recipients of male donor lungs compared to 68.5% of recipients of female donor lungs had any grade of PGD on postoperative day 3 ($p < 0.001$).

Estradiol levels vary over time and are associated with the development of PGD in male but not female recipients

Median pre and postoperative plasma estradiol levels were not significantly different in males compared to females. Estradiol levels were compared between males and females at

all three time points. GEE demonstrated a non-significant difference in the trend in estradiol concentration over time between subjects with and without PGD ($p=0.09$) (Figure 1A). When the cohort was stratified by recipient gender, GEE analysis demonstrated no significant difference in the change over time of estradiol level among female subjects (Figure 1B) ($p=0.9$) but did show a significant difference between male subjects (Figure 1C) with PGD and those without PGD ($p=0.049$).

24-hour postoperative estradiol levels are associated with grade 3 PGD

To test whether circulating estradiol levels were associated with the development of PGD, we compared estradiol levels at all three time points between patients who did or did not develop grade 3 PGD at any time in the 72 hours after transplantation. There were no differences between preoperative or 6-hour postoperative estradiol levels in patients who did versus those who did not develop grade 3 PGD. However, 24-hour postoperative estradiol levels were significantly higher in patients with any grade 3 PGD in both men and women (Figure 2A). When the data were analyzed by gender of the recipient, this association was only significant in male patients (Figure 2B). The quartile of estradiol level at the 24-hour postoperative time point was strongly associated ($p=0.003$) with grade 3 PGD in the 72 hours following transplant (Figure 3A). The 4th quartile has an odds ratio for the development of PGD of 7.9 (95%CI 1.9–33.4, $p=0.005$) versus the first quartile. There was no significant association of estradiol quartile in female patients with PGD while there was a strong association in male patients (Figure 3B). In fact, no male patients in the lowest estradiol quartile developed PGD.

Severity of PGD is associated with estradiol levels at 24 hours postoperatively

Higher estradiol levels at 24 hours postoperatively were associated with greater severity of PGD on day 1 (Figure 4A). Patients with grade 3 PGD had a median (IQR) plasma estradiol level of 77.4 pg/ml (62.5–101.1) versus those without PGD on day 1 who had a median estradiol level of 59.6 pg/ml (45.9–70.6). When the data were analyzed by recipient gender, the association between severity of PGD and estradiol was only present in males (Figure 4B).

Estradiol levels are elevated in patients who underwent cardiopulmonary bypass

40% of patients who were on CPB during the transplantation procedure developed grade 3 PGD in the first 72 hours compared to 18% of the patients who were not on CPB ($p=0.01$). To determine if CPB affected 24-hour postoperative estradiol levels we compared estradiol levels in patients who did not receive CPB during transplantation (median estradiol 60.6 pg/ml, CI 23.7–207.1) to those that did receive CPB (median estradiol 76.1 pg/ml, CI 40.4–219.3) (Figure 5) and found that patients on CPB had higher 24-hour postoperative estradiol levels than patients who were not on CPB ($p=0.024$) (Figure 5A). However, there were no differences in estradiol levels in males versus females according to whether or not they received CPB (Figure 5B).

Multivariable analysis of estradiol levels and PGD status

To determine if any demographic, clinical or process of care variables were influencing the association of estradiol with PGD we did a multivariable logistic regression for the development of grade 3 PGD in the first 72 hours using 24-hour postoperative log-transformed estradiol levels (Table 3). In the unadjusted analysis, there was an odds ratio of 3.1 (CI 1.3–7.6) for the development of grade 3 PGD according to 24-hour postoperative estradiol level. The odds ratio represents the odds of one incremental change in the natural log transformed estradiol level. None of the variables in the adjusted analysis significantly

altered the relationship between 24-hour postoperative estradiol levels and the development of PGD. Adjusting for study site did not affect the multivariable analysis.

Discussion

The primary finding of this study is that estradiol levels measured 24 hours after lung transplantation are strongly associated with the development of grade 3 PGD in male but not female lung transplant recipients. This finding is distinctly different from the critically ill trauma patient population where females have higher post-injury estradiol levels compared to males. (13, 23) Even more intriguing is that the relationship between estradiol levels and increased risk of PGD is seen only in males. This is the first study of its kind to show such an association. Contrary to our hypothesis that female gender would be associated with the development of PGD there was no statistically significant differences in rates of PGD between females and males in our cohort. In addition, higher estradiol levels measured 24 hours after lung transplantation were associated with the development of grade 3 PGD, but only in male and not female lung transplant recipients. Why might estradiol levels be associated with PGD and why is this association seen only in males?

Several animal studies have attempted to define the role of estradiol in the development of acute lung injury with some conflicting results. The specific effects of estradiol in the lung may be dependent on the nature of the lung injury and the timing of the insult. Female mice are more susceptible to the effects of bleomycin-induced lung fibrosis than are male mice (24) and the differences between the sexes disappear when female mice are ovariectomized prior to bleomycin treatment. However, estrogen is protective in a mouse model of lung inflammation induced by intra-tracheal lipopolysaccharide administration. (25) In a mouse model of cystic fibrosis, male mice treated with estrogen had increased severity of *Pseudomonas aeruginosa* pneumonia compared to untreated males and that this was due in part to increased inflammation in male mice (26) suggesting that acute exposure of males to high estrogen levels is pro-inflammatory. Estrogens are also important in lung development and have different effects in males and females. Inhibition of estrogen in utero impairs lung development in male but not female piglets primarily through inhibition of lung alveolarization. (27) These studies suggest that the specific effects of estradiol in the lungs of males and females are variable and may depend on the mechanism of lung injury, making generalizations about estrogen effects in the lung difficult.

One possible mechanism by which estradiol may mediate the development of PGD is through effects on alveolar fluid clearance. We have previously reported that females have faster rates of alveolar fluid clearance (AFC) compared to males with ALI. (13) Furthermore, intact alveolar fluid clearance mechanisms have been associated with better clinical outcomes in patients with severe PGD (28) as well as patients with non-transplant related ALI (29) and patients with severe hydrostatic pulmonary edema. (30) Lung epithelial sodium channels (specifically ENaC) are critical for the resolution of pulmonary edema. In rats, ENaC expression was modulated by estrogens with females having higher levels of ENaC expression in the lung compared to males. (31) Treatment with estrogen and progesterone increased transcription of the ENaC protein in the rat lung but treatment with estradiol alone actually decreased mRNA expression of all 3 ENaC subunits. It is possible that males in our study have lower baseline levels of ENaC expression compared to females and that exposure to high levels of estrogen post transplantation actually resulted in a further decrease in ENaC and thus reduced the rate of alveolar fluid clearance in males.

There are other possible mechanisms by which estradiol may influence the development of PGD in males. One possibility is through modulation of vascular tone and permeability. Estrogens have complex effects on the vasculature that depend on the vascular bed exposed.

Peripheral arteries vasodilate in response to estrogen while pulmonary arteries vasoconstrict in response to estrogen. (32) A recent study showed that elevated levels of circulating endothelin, a potent vasoconstrictor, in lung transplant recipients was associated with worse primary graft dysfunction (33) suggesting that vasoconstriction may be an important factor in the development of PGD. Another study showed that elevated levels of vascular endothelial growth factor (VEGF), a mediator of vascular permeability, predicted the development of PGD in lung transplant recipients. (34) Neither of these studies examined the effects of gender. Another possibility explanation is the effect of estradiol on inflammation. Animal studies have shown pro-inflammatory effects of estrogens (26) and some have looked at the relationship to gender (35, 36) and shown that males may be more susceptible to the proinflammatory effects of estrogens.

Our study cannot differentiate a detrimental effect of estrogen in males from a protective effect of estrogen in females. In this study, male and female transplant recipients had similar levels of estradiol at all three time points. Our data showing that estradiol quartile is associated with the development of grade 3 PGD in a dose dependent manner in males but not in females suggests that in this patient population, estradiol might have a harmful effect in males. Furthermore, estradiol level is also associated in a dose dependent manner with the severity of PGD on the first postoperative day in males but there is no association in females. If estradiol were protective in females we would expect a decrease in grade 3 PGD with increasing concentrations of estradiol as well as a decrease in the severity of PGD on the first postoperative day. It is also possible that estradiol is not mechanistically related to the development of PGD and may instead be a marker of disease. The multivariable analysis would suggest that this is not the case as the relationship between estradiol and the development of PGD is independent of multiple clinical factors that are known or hypothesized to be associated with PGD. However, there may be other confounding factors that we have not accounted for.

We also found that estradiol levels are higher in patients that were placed on cardiopulmonary bypass (CPB) during their transplant procedure. Use of CPB could explain the effects of estradiol seen in our cohort since CPB has been associated with the development of PGD in other studies. (5, 15, 17, 37) [JB1] Although in our study there was an association between CPB and the development of PGD in univariate analysis, 24-hour postoperative estradiol levels were still associated with the development of PGD when controlling for CPB in the multivariable analysis. There was no difference in the percentage of male and female patients who were placed on CPB during transplantation. More patients with pulmonary hypertension were placed on CPB during transplantation (3 patients received CPB while 1 did not) but the very small number of pulmonary hypertension patients in this cohort does not explain these differences. No other diagnosis was over-represented in the CPB group. In older patients (mean age 70 yrs) undergoing coronary artery bypass grafting with CPB, estradiol levels increased in both males and females following surgery (38) but the relationship to clinical outcomes was not measured. Additionally, males may be more sensitive to the cardiovascular effects of estrogens. Higher levels of estradiol levels have been associated with an increased risk of stroke, (39) severity of coronary artery disease (40) and elevated total cholesterol (41) lending credence to the hypothesis that males respond differently to estrogens compared to females. Furthermore, the association of elevated levels of estradiol with diseases of the cardiovascular system in men suggest that men may have more severe endothelial dysfunction in response to estradiol compared to females. In some circumstances, estrogens can have protective effects on the endothelium but this response may be altered in males. We speculate that the elevated levels of estradiol following CPB are a marker of severity of illness and may have different effects in males and females but CPB alone does not explain the effects of estradiol on the development of PGD in males.

This study has some limitations. First, we have no information about exogenous estrogen administration or menstrual cycle in female patients. This could potentially mask an effect of estrogen in females if the effect were specific to a certain phase of the menstrual cycle. Studies have shown that females with asthma actually have a peak in their symptoms in the peri-menstrual period (42) so it is possible that the effects of estrogen in females are cycle dependent. Second, not all patients had blood drawn at all three time points. Given that only postoperative estradiol levels were associated with outcomes, this is unlikely to affect the findings. There is also the possibility of misclassification of PGD grade. PGD grade was assessed by 2 independent investigators according to strict criteria (21) and there was good agreement between the two observers with a kappa statistic of 0.95. However, since the postoperative estradiol levels were associated with the development of PGD in males using multiple different analysis methods the potential for misclassification is unlikely to significantly alter the results. In addition, the majority of the transplant recipients in this study are Caucasian (83%) and the results may not be generalizable to patients of all races. However, it should be noted that racial composition of the study population is representative of the lung transplant recipient population in that the latest report from the Organ Procurement and Transplantation Network in the Department of Health and Human Services reports that 87% of lung transplants to date (from 1988 to present) have been in Caucasian recipients. (43)[JB2] Furthermore, our study only shows an association between estradiol levels and PGD and therefore we cannot draw conclusions about causality. Estradiol may simply be a marker of PGD rather than causally related. Finally, while our findings are interesting and hypothesis generating they should not be generalized to the entire lung transplant population and further study is necessary before these findings are generalized or used in clinical practice.

Conclusions

In summary, estradiol levels in male and female lung transplant patients are similar both pre and postoperatively and higher levels of postoperative estradiol in males but not females are associated both with an increased risk of developing PGD and with the severity of PGD on postoperative day one. In addition, estradiol levels were higher in patients who were on CPB during their transplant procedure but this finding does not explain the association between estradiol levels and PGD. Taken together, these findings suggest that there may be different biologic effects of estrogen in the lungs of males and females following lung transplantation. These findings have implications for future studies of PGD as well as other forms of ALI. Potential therapies to prevent or treat PGD need to be evaluated in the context of gender-specific differences in lung transplant outcomes.

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Figure 1. Estradiol levels over time

Longitudinal median estradiol level across the pre-transplant, 6-h post-transplant, and 24-h post-transplant time points **Panel A**, all study subjects; **Panel B**, female study subjects; **Panel C**, male study subjects. Dashed line represents subjects with PGD and solid line represents PGD-free subjects. Error bars represent the 95% CI. P-value reported is from GEE modeling.

Figure 2. Estradiol levels in patients with grade 3 PGD

24-hour postoperative estradiol levels were measured in patients with (n=29) and without (n=72) grade 3 PGD at any time in the first 72 hours following transplant. Having grade 3 PGD was associated with higher levels of estradiol on postoperative day 1 (**Panel A**) $p=0.002$ by Mann Whitney U test. When analyzed by gender of the recipient, (**Panel B**) the difference was only seen in males. $*p=0.001$ by Mann Whitney U test.

Figure 3. Primary graft dysfunction and 24-hour postoperative estradiol quartile

Panel A shows percentage of all patients with grade 3 primary graft dysfunction at any time in the first 72 hours after transplant according to 24-hour postoperative estradiol level. The risk of PGD increases with each quartile of estradiol ($p=0.003$ for trend by linear-by-linear association). Panel B shows the same data by gender. There is no difference in risk of PGD by estradiol quartiles in females ($p=0.299$ for trend by linear-by-linear association) but there is an increase risk in males for each quartile ($p=0.001$ for trend by linear-by-linear association).

Figure 4. Severity of PGD is associated with 24-hour postoperative estradiol level

Panel A shows 24-hour postoperative estradiol level in all patients according to grade of PGD on day 1 ($p=NS$ by Kruskal Wallis test for trend). **Panel B** shows the same data by gender. Estradiol levels do not vary by PGD grade in females but increasing levels are associated with higher grades of PGD in males (** $p=0.014$ by Kruskal Wallis test for trend across all grades, $p=0.007$ by Mann Whitney U test for grade 0 compared to grade 3).

Figure 5. Estradiol levels in patients on cardiopulmonary bypass (CPB)

24-hour postoperative estradiol levels are higher in patients who were placed on cardiopulmonary bypass (CPB) during their transplant (**Panel A**), ($p=0.024$ by Mann Whitney U test). There were no differences in estradiol levels between males ($N=29$) and females ($N=29$) (**Panel B**) who were not on CPB ($p=0.822$) or males ($N=18$) and females ($N=25$) that were on CPB ($p=0.571$).

Table 1
Clinical Characteristics of 111 Lung Transplant Recipients

	Lung Transplant Recipients (n=111)			
	Males (n=53)		Females (n=58)	
	No PGD (n=41)	PGD (n=12)	No PGD (n=39)	PGD (n=19)
Recipient Variables				
Age, years, mean (SD)	53.2 (+/-12)	55.5 (+/-10.6)	51.6 (+/-14.0)	51.3 (+/-12.4)
Caucasian	90%	83%	87%	68%
Diagnosis				
COPD	37%	25%	36%	21%
CF	20%	8%	26%	5%
IPF	41%	42%	33%	42%
IPAH	2%	17%	0%	5%
Single lung transplantation	37%	25%	44%	16%
Cardiopulmonary bypass	39%	58%	33%	68%
Donor Variables				
Age, years, mean (CI)	31.8 (21.5–46.2)	29.1 (25.5–46.3)	34.5 (22.3–49.7)	41.9 (30.4–52.9)
Female	27%	42%	70%	74%
Caucasian	60%	83%	51%	58%

COPD = chronic obstructive pulmonary disease; CF = cystic fibrosis; IPF = idiopathic pulmonary fibrosis; IPAH = idiopathic pulmonary arterial hypertension.

Table 2

Percent of recipient males and females with grade 3 PGD on days 1–3.

	Estradiol cohort (n=111)		
	Male (n=53)	Female (n=58)	P value
Day 1	18.9	24.1	0.645
Day 2	9.4	17.2	0.275
Day 3	13.7	19.0	0.450

Table 3
Multivariable analysis of estradiol levels and primary graft dysfunction

Variable	Odds Ratio for development of PGD by estradiol level	P value
Unadjusted	3.1 (1.3–7.6)	0.012
Adjusted for		
Recipient age	3.2 (1.3–7.8)	0.011
Recipient gender	3.0 (1.3–7.4)	0.014
Recipient race	2.9 (1.1–7.2)	0.027
Donor age	2.9 (1.2–7.0)	0.020
Donor gender	3.3 (1.3–8.1)	0.009
Donor race	3.6 (1.4–9.0)	0.007
Transplant type	3.2 (1.3–7.8)	0.011
PPH diagnosis	3.2 (1.3–7.8)	0.012
CPB	2.7 (1.1–6.7)	0.031
Ischemic time	3.1 (1.3–7.5)	0.013
Intra-OP PASP	3.5 (1.4–8.8)	0.008

PPH = primary pulmonary hypertension, CPB = cardiopulmonary bypass, OP = operative, PASP = pulmonary artery systolic pressure