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Early View

Research letter

Cytomegalovirus – an unrecognised potential contributor to cystic fibrosis disease progression?

Michael D. Parkins, Kathleen J. Ramos, Christopher H. Goss, Ranjani Somayaji

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Cytomegalovirus – an unrecognized potential contributor to cystic fibrosis disease progression?

Michael D. Parkins[1], Kathleen J. Ramos[2], Christopher H. Goss[2], Ranjani Somayaji[1]

[1]Department of Medicine, University of Calgary, Calgary AB CANADA
[2]Department of Medicine, University of Washington, Seattle WA, USA

Take Home Message:
Of cystic fibrosis (CF) patients referred from our centre for lung transplant consideration, those who were cytomegalovirus (CMV) IgG+ at referral were eight years younger then seronegatives, suggesting CMV may have a pathogenic role in CF lung disease.

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Corresponding author:
Michael D. Parkins.
3330 Hospital Drive, NW, Calgary, Alberta, Canada T2N 4N1
Phone: 403-210-7913, Fax: 403-270-2772
e-mail: mdparkin@ucalgary.ca

Abstract:

Cytomegalovirus (CMV) is a common human beta-herpes virus most notable for causing visceral disease in profoundly immune-suppressed populations, and congenital infections. However, an increasing body of work has demonstrated that CMV seropositivity is associated with a number of chronic medical conditions including heart disease and dementia – potentially related to the effects of chronic inflammation. We hypothesized that the outcomes of individuals with cystic fibrosis (CF), a chronic inflammatory disease, could similarly be associated with CMV-status. We performed a single-centre retrospective study of all 71 individuals with CF referred for lung transplantation from our CF centre between 1991-2017 and assessed how CMV serostatus associated with patient pre-transplant outcomes. We observed CMV IgG positivity was associated with disproportionate progression to end-stage lung disease as defined by death/or transplantation in our cohort (27.2 vs 35.1 years, difference 7.95 (95% CI 3.61-12.29 years), $p<0.001$) which remained significant following adjustment for confounders (difference 6.96 (95% CI 2.51 – 11.41 years). CMV may represent a potentially important modifier of CF lung disease, warranting further study.

Plain Language Summary:

Recent data has shown chronic infection with cytomegalovirus (CMV) is associated with more rapid progression of chronic inflammatory diseases including heart disease and Alzheimer's, and is associated with increased risk of death in general populations. How this might occur is unknown – but it is suspected that CMV replicates in already inflamed tissues thereby exaggerating resultant organ damage. As cystic fibrosis (CF) is a disease of infection and inflammation, it is a perfect candidate for exploitation by CMV. Preliminary data from our centre has demonstrated that amongst CF patients referred for lung transplant consideration, those who were CMV positive reached end-stage lung disease eight years earlier than those who were CMV negative – suggesting CMV may be an important modifier of CF disease progression.

Cytomegalovirus (CMV) is a beta-herpes virus, whose impacts are well known to clinicians providing post-transplant CF care. Lung transplant recipients have the highest risk of any solid-organ transplant for CMV reactivation and ganciclovir resistance[1, 2]. Furthermore, CMV reactivation increases the risk of chronic lung allograft dysfunction. However, even in general populations CMV seropositivity is associated with adverse outcomes including; cognitive impairment, frailty, heart disease, and all-cause mortality[3-5]. How CMV may contribute to disease is not evident, but many streams of evidence suggest CMV replication in inflamed sites contributes to exaggerated inflammation and tissue injury[6]. Individuals with cystic fibrosis (CF) experience chronic inflammation within the airways leading to remodelling and eventually respiratory failure. Indeed, inflammatory biomarkers in the sputum and serum of CF patients correlate with short and long-term outcomes[7]. We hypothesized that CMV may represent an unrecognized contributor to CF lung disease.

We performed a detailed chart review of all Calgary Adult CF Clinic patients who were referred for lung transplantation, where CMV IgG testing would be performed. CMV serostatus, demographics, infecting pathogens, markers of nutrition and lung function from last clinical encounter were recorded for those who were transplanted or succumbed to disease. Our primary outcome was a composite end-point of age at lung transplantation/death without transplant. We also analysed the outcomes of death and lung transplant separately by CMV serostatus, and conducted a stratified analysis per time period to account for improvements in care (1990-1999/2000–2009/2010–2017).

Socio-demographic and clinical characteristics including referral and listing for transplantation were summarized. Univariate and multivariable linear regression models were constructed for the primary outcome to compare CMV+ and CMV- patients. We incorporated clinical (disease severity and microbiology) factors that varied significantly between patients by CMV status as well as sex, body mass index (BMI) and educational status (less than high school/high school/technical college/university) (which trended toward significance and were biologically plausible). Non-parametric tests of trend were conducted to compare outcomes between periods. . Significance was based on $\alpha<0.05$, all hypothesis tests were 2-sided and statistical analysis was performed using Stata V14.2(College Station, USA).

Since 1991, 71 patients (50.7% female) were referred and listed for transplant consideration who either successfully received a life saving transplantation or died waiting. Of these, 59 received bilateral lung transplants (83%) and 12 (16.9%) died prior transplantation. Of the cohort, 15 patients were excluded (6 died, 9 transplanted) from the analysis as they did not have CMV serology documented. Patients who were excluded were demographically similar, but were more likely to represent earlier cohorts (44% 1990-1999; 14% 2000-2009; 0% 2010-2017, p=0.004).

Of the 56 included, 30 (54.6%) were CMV+ and the prevalence of CMV seropositivity did not differ by time-period. When socio-demographic and clinical characteristics were examined, patients who were CMV- were more likely to be F508del homozygous and have *B. cepacia* complex infection, but otherwise did not differ from CMV+ individuals(Table 1). Lung transplantation occurred a median of 1.25 years (IQR 0.87-1.78) after referral and 0.44 years following listing (0.14-0.90) and did not differ by CMV status.

For the primary outcome, patients who were CMV+ died or underwent bilateral lung transplantation at a significantly younger age compared to those who were CMV- (27.17 vs 35.11 years, difference 7.95 (95% CI 3.61-12.29 years), $p<0.001$). The difference in age at death or lung transplant remained significant in a multivariable model adjusted for sex, BMI, *B. cepacia* infection, genotype and education (6.96 years, 95% CI 2.51 to 11.4 years, $p =0.003$). When the outcomes of death and transplant were assessed separately, CMV+ was associated with a significantly lower mean age at either death (difference 9.35 years (95% CI 0.89 – 17.82), $p = 0.03$) or lung transplantation (difference 7.36 years (95% CI 2.58 – 12.13, $p = 0.003$). No significant differences in age at death or transplant were noted by CMV serostatus ($p>0.05$) when outcomes were stratified by time periods.

Our retrospective study demonstrated a significant age disparity at death/lung transplantation in persons with CF suggesting a deleterious association for CMV. However, we must consider a number of limitations to these observations. Most importantly, association does not confirm causation[8]. This was at a single-center study with a limited sample size and limited power to detect potentially meaningful differences in the outcome in the stratified analyses. Although there was no evidence of changing rates of CMV seroprevalence, the study spanned ≥25 years and considerable improvements in CF outcomes have since been realized. However, when we analyzed our outcomes stratified by time periods, no significant changes in the association were observed. A portion of patients were excluded from analysis due to missing CMV status leading to potential selection bias, but they were demographically similar to the analyzed cohort. While we were able to identify CMV serostatus of patients at the time of transplant referral, we were unable to determine when patients were infected, and infection duration may have differential effects on clinical outcomes. Within general populations, CMV seropositivity increases over time with rates increasing from 36% in those 6-11 years, 49% 20-29 years, 65% at 40-49 years and >91% in those >80 years[9]. Accordingly, duration of CMV infection may be a more important predictor of progression to the primary outcome as opposed to serostatus at a single time point. Furthermore, quantitative levels of CMV IgG may be more sensitive at identifying risk as opposed to current reporting of qualitative results[4]. CMV seropositivity is known to disproportionally exist in socially marginalized groups[10]; socioeconomic status is an important modifier of CF[11] and influences access to lung transplant referral and listing[12]. However, we did not identify significant differences between groups in socioeconomic status using education and income indicators.

Our knowledge of CMV pathogenesis offers a potential model through which CMV may accelerate disease progression in CF; i). The lungs are a major site of CMV reactivation[13] and progressive lung disease is responsible for the majority of CF morbidity and mortality. ii). CMV reactivation can be triggered by bacterial infection[14] – bacterial infection is omnipresent in CF. iii). CMV reactivation is associated with reduced immune surveillance[15] and thusly increased susceptibility to infections. iv). CMV reactivation is associated with an exaggerated immune response[16] – potentially enhancing tissue damage. CMV infection could partially explain the varied clinical courses experienced by individuals with CF and why conventional microbiologic markers poorly correlate with pulmonary exacerbation recovery and long-term outcomes[17].

To confirm if CMV might associate with disease progression in CF we can leverage CMV serostatus data collected as part of transplant databases (e.g. the International Society for Heart & Lung Transplantation Registry) in efforts to understand CMV pathogenesis pre- and post-transplantation - to confirm the results of this single centre study. Furthermore, cross-sectional seroprevalence studies of CF general populations captured with national registries will enable us to correlate critical markers of CF disease progression with CMV status such as baseline lung function, changes in lung function and exacerbation frequency. We would advocate for prospective longitudinal studies of CF populations to assess for factors associated with incident infections/seroconversion in order to determine if acquisition of CMV infection is associated with change in disease status and how infection duration correlates with clinical outcomes. Indeed, a case-series of three individuals experiencing exacerbations in the context of CMV seroconversion was recently published[18]. Finally, exploring the role of CMV reactivation/viremia and its association with exacerbation occurrence and recovery and rates of lung function decline is important, as demonstrated by efforts currently underway to explore CMV pathogenesis in intensive care units[19].

The identification of CMV's association with CF lung disease progression could have tremendous implications for disease management. If this association is confirmed in larger multi-centre studies, strategies exploring the use of pre-emptive and prophylactic anti-viral treatments, including acyclovir (while not having significant CMV activity can reduce reactivation potential) or valganciclvoir, in specific populations may be in order[20]. Considerable progress in developing an effective vaccine for CMV has been made recently – and a large number of products are currently being investigated[21]. The rationale for these vaccines has predominately focused on their role in preventing congenital cases of CMV and avoiding transplant complications in pre-transplant patients. However, it may be that individuals with CF who are seronegative for CMV may derive benefit from effective vaccine strategies both acutely and in the long-term setting of their risk for transplant.

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Table 1: Characteristics of cohort at time of referral to lung transplantation consideration

Factor	CMV (-) (n=26)	CMV (+) (n=30)
Female	12 (46%)	17 (57%)
F508homozygous	19 (76%)	13 (43%)
≥1 F508 allele	23 (92%)	21 (70%)
Pancreatic insufficiency	24 (96%)	27 (90%)
CF-related diabetes	6 (24%)	10 (33%)
CF-liver disease	6 (24%)	4 (13%)
CF-arthropathy	5 (20%)	4 (13%)
CF-sinus disease	12 (48%)	15 (50%)
Recurrent DIOS	9 (36%)	14 (47%)
GERD	17 (68%)	18 (60%)
FEV_1 (% predicted)	21 (IQR 18-27)	25 (IQR 21-30)
FVC (% predicted)	50.5 (IQR 42-58)	44.5 (IQR 38-49)
BMI (kg/m^2)	20.5 (IQR 18.5-22.0)	19.7 (IQR 17.6-20.8)
Receipt of enteral nutrition	14 (58%)	12 (40%)
Supplemental Oxygen	24 (96%)	28 (93%)
Litres/min oxygen	3 (IQR 3-4)	4 (IQR 3-5)
Pseudomonas aeruginosa	22 (88%)	28 (93%)
Bcc	6 (24%)	1 (3%)
Stenotrophomonas maltophilia	2 (8%)	3 (10%)
MSSA	7 (28%)	8 (27%)
MRSA	1 (4%)	0
Achromobacter spp.	0	1 (3%)
Aspergillus	6 (24%)	5 (17%)
Did not complete high school	5 (20%)	9 (30%)
At least high school	7 (28%)	12 (40%)
Diploma	6 (24%)	2 (7%)
University	7 (28%)	7 (23%)

* DIOS=distal intestinal obstruction syndrome, GERD=gastroesophageal reflux, FEV_1 = forced expiratory volume in one second, FVC=forced vital capacity, Bcc= *Burkholderia cepacia* complex, MSSA= methicillin susceptible *Staphylococcus aureus*, MRSA = methicillin resistant *S. aureus*