Institution: University College London

Unit of Assessment: 1 - Clinical Medicine

Title of case study: Development and validation of a biomarker for cytomegalovirus disease after transplant improves identification of patients at risk, speed of diagnosis and treatment

1. Summary of the impact

Basic and translational research undertaken since 1993 by UCL Virology has defined the natural history and pathogenesis of cytomegalovirus (CMV) infection and disease. As a consequence of our work, rapid diagnosis and pre-emptive therapy are now available worldwide for this important infection. We have provided a national reference service for strains of CMV resistant to current antiviral drugs and for diagnosis of congenital CMV infection.

2. Underpinning research

Cytomegalovirus (CMV) is a very common virus infecting 60-100% people worldwide. In healthy individuals it usually goes unnoticed, but in certain immunocompromised groups it can be fatal. It is the most frequent cause of intrauterine infection, and causes permanent disability in 1-2 live births per thousand. It is also the commonest infectious agent to affect transplant patients, and is implicated in causing death in other at-risk populations, specifically elderly patients and those with HIV infection.

Research at the UCL Department of Virology led by Professor Paul Griffiths developed assays to measure CMV DNA in infected humans using non-nested polymerase chain reaction (PCR) amplification of the UL55 gene. We showed that CMV replicated rapidly in humans (with a doubling time of approximately one day) so establishing the schedule of monitoring of CMV infection after transplantation **[1]**. We also used these assays to show that measurements of viral load acted as prognostic markers for end-organ disease **[2, 3]**. These studies of natural history of CMV infection were extended to other cohorts of patients, where CMV viraemia was significantly associated with mortality in AIDS patients **[4]**. The work demonstrated the high inherent pathogenicity of CMV, and the role of the immune system in controlling infection even in patients who were immunocompromised. Our assays of viral replication have been used to measure the effectiveness of pre-emptive antiviral therapy, which identifies early viral replication after transplantation which then triggers antiviral therapy (the alternative is to treat all transplants with antiviral drugs regardless of whether they are infected or not). Our recent data have generated reassuring outcomes that this approach achieves good control of CMV disease after liver and kidney transplantation **[5].**

We also proposed that the virus persisted in sanctuary sites within the body protected by immune evasion genes that resulted in a sub-optimal immune response. We concluded that it would be necessary to improve natural immunity to the virus. To do this we conducted a randomised controlled trial of a prototype CMV vaccine, showing that vaccination provided substantial control of the viral load in patients undergoing liver and kidney transplantation **[6]**. Importantly, natural immunity in these immunocompromised hosts was boosted when vaccine was given to people with natural infection. The amount of antibody made in response to the vaccine correlated with the protection observed indicating that humoral immunity is important in patients with impaired cell mediated immunity. We also developed new PCR assays to detect CMV DNA in dried blood spots obtained routinely at birth and use them to provide a national service for retrospective diagnosis of congenital CMV infection when children present with sensorineural hearing loss or developmental delay **[7]**.

3. References to the research

[1] Emery VC, Cope AV, Bowen EF, Gor D, Griffiths PD. The dynamics of human cytomegalovirus replication in vivo. J Exp Med. 1999 July 19;190(2):177-82.

http://dx.doi.org/10.1084/jem.190.2.177

[2] Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet . 2000 June 10;355(9220):2032-6. http://dx.doi.org/10.1016/S0140-6736(00)02350-3

[3] Emery VC, Griffiths PD. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. Proc Natl Acad Sci U S A. 2000 July 5;97(14):8039-44. http://dx.doi.org/10.1073/pnas.140123497

[4] Deayton JR, Sabin CA, Johnson MA, Emery VC, Wilson P, Griffiths PD. Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet. 2004 June 26;363(9427):2116-21. http://dx.doi.org/10.1016/S0140-6736(04)16500-8

[5] Atabani SF, Smith C, Atkinson C, Aldridge RW, Rodriguez-Perálvarez M, Rolando N, Harber M, Jones G, O'Riordan A, Burroughs AK, Thorburn D, O'Beirne J, Milne RS, Emery VC, Griffiths PD. Cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy. Am J Transplant. 2012 Sep;12(9):2457-64. http://dx.doi.org/10.1111/j.1600-6143.2012.04087.x.

[6] Griffiths PD, Stanton A, McCarrell E, Smith C, Osman M, Harber M, Davenport A, Jones G, Wheeler DC, O'Beirne J, Thorburn D, Patch D, Atkinson CE, Pichon S, Sweny P, Lanzman M, Woodford E, Rothwell E, Old N, Kinyanjui R, Haque T, Atabani S, Luck S, Prideaux S, Milne RS, Emery VC, Burroughs AK. Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial. Lancet. 2011 Apr 9;377(9773):1256-63. http://dx.doi.org/10.1016/S0140-6736(11)60136-0.

[7] Walter S, Atkinson C, Sharland M, Rice P, Raglan E, Emery VC, Griffiths PD. Congenital cytomegalovirus: association between dried blood spot viral load and hearing loss. Arch Dis Child Fetal Neonatal Ed. 2008 Jul;93(4):F280-5. http://dx.doi.org/10.1136/adc.2007.119230

4. Details of the impact

We have developed PCR assays which have been adopted into clinical practice to allow rapid diagnosis of CMV viral load, and have been used to initiate pre-emptive therapy with anti-viral drugs. In addition, they are used to provide a national service for retrospective diagnosis of congenital CMV infection. Our work has also influenced the development of vaccines by the pharmaceutical industry.

Development of assays and introduction into clinical practice

The underpinning research described above has resulted in a new understanding of the natural history and pathogenesis of CMV infection, which has been applied in multiple ways for patient benefit. Assays were developed for rapid quantitative diagnosis and were promptly introduced into routine clinical practice by our group. These assays were patented and licensed from 2006 by the Health Protection Agency, meaning that CMV rapid diagnosis and pre-emptive therapy are now available throughout the UK. Between 2008 and 2010 almost 19,000 tests were performed, generating royalties to UCL of *[Text removed for publication]* **[a]**. Our research on the usefulness of detecting CMV DNA is cited in the latest guidelines on managing CMV post-transplantation **[b]**.

Pre-emptive treatment in transplantation

Pre-emptive treatment is now widely used with virtually all bone marrow transplant patients worldwide. This reduces the risk to the bone marrow from anti-CMV drugs **[c]**. In solid organ transplants, most centres still administer prophylactic antivirals to all patients regardless of whether they have CMV infection. However the latest guidelines from the USA have moved from preferring prophylaxis to equipoise, citing our recent results demonstrating the efficacy of pre-emptive

therapy **[b]**.

Diagnosis of congenital CMV infection

Our PCR assays are used to provide a national service for retrospective diagnosis of congenital CMV infection when children present with sensorineural hearing loss or developmental delay, as recommended by two sets of guidelines issued by the British Association of Audiovestibular Physicians in 2009 **[d]**. Since 2008, 1,455 tests have been performed using our method **[e]**.

Development of CMV vaccines

Our work has stimulated investment by several pharmaceutical companies in prototype CMV vaccines which are currently entering clinical trials. Research at UCL has provided much of the scientific basis for its current control by means of antiviral therapy and what we hope will be its ultimate elimination by means of routine immunisation **[f]**. At a national level, the Department of Health Joint Committee on Vaccination and Immunisation invited vaccine manufacturers to submit evidence in 2012 about their plans for preparing CMV vaccines as part of the committee's horizon scanning **[g]**. At an international level, our findings have been presented to the two USA Federal meetings which have reviewed the prospects for developing CMV vaccines (CDC/NIH 2000 Atlanta; FDA/CDC/NIH/NVPO Washington DC 2012) **[h]**. We also provided evidence to the Decade of Vaccines consortium which produced a Global Vaccine Action Plan proposing that, for the future, vaccines should be prepared against four virus infections which are currently not vaccine-preventable. One of these is CMV and the proposal was endorsed by the World Health Assembly in 2012 **[i]**.

Raising awareness of CMV: public and patient engagement

In 2012, Griffiths worked with patient support group CMV Action to produce a summary of current UK guidelines aimed at parents of children with congenital CMV. This document set out key points from the guidelines to help families to understand what tests their child will have and why **[j]**. Griffiths has also written a book on CMV entitled *The Stealth Virus* which aimed to explain CMV to the non-specialist **[k]**. This book was welcomed by CMV Action and recommended on patient websites **[l]**.

5. Sources to corroborate the impact

[a] Corroboration of licensing and numbers of tests can be obtained from UCL Business. Contact details provided.

[b] Kotton CN, Kumar D, Caliendo AM, Asberg A, Chou S, Danziger-Isakov L, Humar A; Transplantation Society International CMV Consensus Group. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2013;96:333-60. http://dx.doi.org/10.1097/TP.0b013e31829df29d. *Our work on CMV detection is cited on page 336, on CMV vaccination on page 340 and on pre-emptive therapy on page 341.*

[c] Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood. 2009;113 :5711-9. http://dx.doi.org/10.1182/blood-2008-10-143560

[d] "Aetiological Investigations into bilateral mild to moderate permanent hearing loss in children" and "Aetiological Investigations into bilateral severe to profound permanent hearing loss in children", both of which cite our work with regard to testing for CMV. http://www.baap.org.uk/index.php?option=com_content&view=article&id=48&Itemid=54

[e] Royal Free Pathology. Contact details of Lab Manager provided.

[f] Griffiths P, Plotkin S, Mocarski E, Pass R, Schleiss M, Krause P, Bialek S. Desirability and feasibility of a vaccine against cytomegalovirus. Vaccine. 2013 Apr 18;31 Suppl 2:B197-203.

http://dx.doi.org/10.1016/j.vaccine.2012.10.074.

[g] http://cmvaction.org.uk/experts-positive-about-the-future-of-a-cmv-vaccine/

[h] http://videocast.nih.gov/summary.asp?live=10857

[i] http://www.dovcollaboration.org/dov-collaboration-updates/world-health-assembly-endorses-new-plan-to-increase-global-access-to-vaccines

[j] CMV Action. CMV: What support to expect. Sept 2012. http://cmvaction.org.uk/wp-content/uploads/2011/09/cmv-expect-A4-oct12-1.pdf

[k] www.amazon.co.uk/Stealth-Virus-Prof-Paul-Griffiths/dp/1477566791

[l] CMV Action: http://cmvaction.org.uk/new-book-on-cmv-aimed-at-raising-awareness/
The Baby Website:
http://www.thebabywebsite.com/article.132.Pregnancy_and_cytomegalovirus_%28CMV%29.htm