Diagnosis of cytomegalovirus pneumonitis following stem cell transplantation: addressing the Yin and Yang of molecular methods.

In the early days of allogeneic stem cell transplantation, pneumonitis caused by cytomegalovirus (CMV) was a feared complication with a high mortality whose diagnosis could only be made by lung biopsy.(1) The occurrence of disease once the marrow had engrafted, coupled with interstitial infiltration of the lungs with lymphocytes, suggested the possibility of an immunopathological component to the pathogenesis, triggered by the presence of CMV.(2) Simply detecting CMV infection in the lungs may not diagnose current CMV pneumonitis with high specificity, yet may be a harbinger for its future development in some patients.(3)

Once ganciclovir became available, investigators sought evidence of active CMV infection in the lungs by using bronchoalveolar lavage and processing the samples with the then new technology of cell culture confirmation based on monoclonal antibodies specific for CMV.(4, 5) In a pioneering study in 1991, researchers sampled the lungs on day 35 post-transplant in 104 patients and again later in some of them.(6) When CMV was detected at day 35, patients were offered randomization to standard care or to receive intravenous ganciclovir in addition. Receipt of this drug significantly reduced the primary endpoint of death or CMV pneumonitis from 70% (14/20) to 25% (5/20). In an accompanying editorial, Dr Bob Rubin coined the term pre-emptive therapy to describe the strategy of detecting CMV infection early post transplant and treating it before it evolved into serious disease.(7) His inspiration for this terminology came from watching live television broadcasts showing cruise missiles destroying ground radar systems so that death from subsequent manned conflict could be reduced during the First Gulf War. Thus, that war and the term pre-emptive therapy both celebrated their 25th anniversaries last year.(8) While the clinical trial took a major step forward, it was not sufficient to control all cases of CMV disease, because the study also reported that repeated sampling for CMV infection would be required to identify all patients destined to develop CMV pneumonitis.(6) There were obvious practical difficulties to performing serial BALs but, fortunately, the description of polymerase chain reaction (PCR) allowed CMV DNA to be detected in blood.(9) Monitoring stem cell transplant patients serially for DNAemia thus replaced routine day 35 bronchoalveolar lavage, although the term, pre-emptive therapy, was retained.

In those days, PCR for CMV DNA was qualitative. Research assays showed a link between the quantity of CMV in the blood (CMV viral load) and CMV end-organ disease, but were not applicable to routine clinical diagnosis.(10) Around 2003, real-time PCR became available and was quickly taken up by many laboratories to diagnose CMV DNAemia. Just as PCR became useful for testing blood, it was also widely adopted for other samples including BAL.(11) This meant that the classical techniques of traditional cell culture with cytopathic effect as a readout, or monoclonal antibodies for cell culture confirmation, were less likely to be requested. With time, these older assays disappeared from the repertoire of diagnostic laboratories. There was clear patient benefit from the availability of rapid molecular diagnosis because this

undoubtedly was one factor that contributed to the decreasing incidence of CMV pneumonitis (reviewed in (1)).

The Yang that corresponds to this Ying is the absence of data to document the sensitivity and specificity of PCR when different viral loads are detected in BAL. This is a concern, because these immunocompromized hosts do not suffer from individual opportunistic infections one at a time; multiple infections may be present, only some of which contribute to pathogenesis. Available treatments also have important side effects, such as the bone marrow toxicity of ganciclovir, so accurate diagnosis is required to guide therapy in these complex patients who are already receiving multiple drugs. Furthermore, definitive diagnostic criteria are required as strict endpoints for clinical trials of newer antiviral drugs and vaccines with potential activity against CMV.(12)

How could clinical researchers address this problem? No one would advocate returning to definitive diagnosis through invasive lung biopsy in this group of vulnerable patients. Case series and collective clinical experience of BAL coupled with clinical diagnoses can accumulate and be shared, but can only provide the weakest type of scientific evidence. Current clinical guidelines that recognize the need for robust diagnosis for confirmed cases of CMV pneumonitis, state(12) that "detection of CMV by PCR alone may be too sensitive for the diagnosis of CMV pneumonia." This statement is prescient, for this issue of the journal contains much-needed quantitative data.(13)

Researchers at Fred Hutchinson Cancer Centre in Seattle reviewed their case series of CMV pneumonitis in 132 patients post stem cell transplant. The quantitative PCR results from the clinically diagnosed cases of CMV had a median $\log_{10}$ of 3.9 (IQR 2.6-6.0) IU/ml BAL fluid. A control group was provided by 118 stem cell transplant patients whose pneumonia was attributed to infectious agents other than CMV. The quantitative PCR results from these patients with non-CMV pneumonia showed a median $\log_{10}$ viral load of 0 (IQR 0-1.6) IU/ml. Importantly, the researchers provide another control group; patients who volunteered in 1988-89 to be part of a natural history study by undergoing BAL at day 35-45 post stem cell transplant.(3) The median $\log_{10}$ viral load in these controls was 1.6 (IQR 0-2.5) IU/ml. Both groups of control patients were at risk of CMV infection by virtue of recipient or donor seropositivity pre-transplant. Thus, CMV DNA can be detected at low levels in BAL of some stem cell patients with pneumonia attributed to other infections or in those without overt disease. However, the levels so low that they can be distinguished from the higher levels found in cases of true CMV pneumonitis.(13)

To critically assess the diagnostic value of these quantitative results, the authors constructed receiver operator curves from the values in BAL samples obtained before treatment was started. The positive predictive values and negative predictive values are given for particular levels of CMV DNA found in BAL, with a suggested optimum cut-off of 500 IU/ml. The results are analyzed and presented in multiple ways to allow individual transplant centers to set their diagnostic cut-off values to suit the baseline characteristics of their patients and expected prevalence of CMV pneumonitis. In addition, they show

that the results are not affected by the presence of co-pathogens or pulmonary hemorrhage. Portability of the results between centers is facilitated by expressing the PCR values in international units/ml of BAL fluid.(14) The results also bring to mind a new design for a randomized controlled trial. Once the value of 50 IU/ml is accepted as the cut-off to initiate therapy with ganciclovir and immunoglobulin, patients with lower levels could be randomized to receive an anti-CMV drug or placebo to see if future pneumonitis could be prevented. At the moment, such a study would have the disadvantage of giving a drug that is toxic to bone marrow, but the availability of one of three new compounds that lack such side-effects would change the risk-benefit calculation.(15-17)

Overall, this paper is a tour de force that illustrates the importance of incorporating natural history studies into translational research projects. The heroes of this paper are arguably the patients who selflessly volunteered to have BAL performed 35-45 days after their stem cell transplant. The results in these altruists have elevated this report from a standard well-controlled case series to a seminal publication that will be widely used and cited, thus illustrating the benefits for both science and medicine of clinically-relevant academic health research.

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