20. **Comparison of cyclosporine assays using radioimmunoassay, fluorescent polarization immunoassay and high-performance liquid chromatography**


**Introduction**

We have investigated three different methods for the assay of blood levels of cyclosporine to compare the results obtained by the different assays and to see which assay is the most suitable for routine use in our laboratory.

**Material and methods**

The following commercially prepared kits were used.

1. Sandimun cyclosporine radioimmunoassay using a monoclonal antibody that specifically measures parent cyclosporine (SP. RIA).
2. Sandimmun cyclosporine radioimmunoassay using a monoclonal antibody that measures both parent drug and metabolites (non-SP. RIA).
3. ABBOT TDX fluorescent polarization immunoassay (FPIA) that measures parent cyclosporine and its metabolites.
4. A local modified high-performance liquid chromatography assay (HPLC) using solid phase extraction that measures parent cyclosporine.

Peripheral blood was collected from 50 patients attending our outpatient clinic in one day to measure the trough levels of cyclosporine. The commercially available cyclosporine assay kits were used in accordance with the manufacturer's instructions.

The HPLC assay is summarized as follows: 4 ml of acetonitrile (ACN), methanol and 10% zinc sulphate solution is added to a spiked blood sample and the denatured proteins separated by centrifugation. The supernatant is diluted with deionized water and the mixture added to a 6 ml c-18 bond elute cartridge. The cartridge is washed with 50% acetonitrile in water and the cyclosporine eluted with 2 ml of ethanol into a clean tube. The eluent is evaporated to dryness under nitrogen at 45 °C and then the residue reconstituted with 180 μl of ACN, methanol and water. The samples are injected into a
C-1-Reverse phase column at 58 °C with a flowrate of 1 ml/min. The column eluents are monitored at 214 nm using a detector.

The assays were compared by calculating the correlation coefficient and plotting a linear regression line.

Results

Figures 1 and 2 summarize the comparison between Sandimmun specific and

![Fig. 1. Assay of cyclosporine: HPLC vs Sandimmun RIA (specific).](image1)

![Fig. 2. Assay of cyclosporine: Sandimmun RIA (non-specific) vs Abbot TDX. FPIA (non-specific).](image2)