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File ID 83219
Filename SUMMARY

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type Dissertation
Title Measuring treatment response in HIV-1 infection
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Faculty Faculty of Medicine
Year 2000

FULL BIBLIOGRAPHIC DETAILS:

<http://dare.uva.nl/record/86495>

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SUMMARY

In this thesis several studies are described in which antiretroviral treatment in HIV-1 infection was evaluated. Initially, only clinical endpoints, immunological parameters, and an incomplete measure of viral replication (p24 antigen) were available for this purpose. In the early nineties direct estimation of virological replication became available, by means of HIV-1 RNA assays. In Chapter 2 serum HIV-1 RNA and p24 antigen levels were examined in 28 seropositive asymptomatic individuals participating in a trial on the efficacy of zidovudine. Sixteen individuals remained asymptomatic until 4 years after the onset of the trial, whereas 12 individuals were diagnosed with an AIDS-defining event. The serum HIV-1 RNA load and p24 antigen levels were determined before the onset of therapy and during the first 8 weeks of therapy to establish whether the patterns of change were predictive of clinical outcome. Among the 28 participants 43% had measurable pretreatment concentrations of p24 antigen. Initiation of zidovudine therapy was followed by a similar decline of p24 antigen levels in non-progressors as well as progressors and, therefore, these groups could not be distinguished on the basis of this parameter. HIV-1 RNA was detected in the pretreatment samples of 82% of the individuals and could be detected in p24 antigen-positive as well as p24 antigen-negative individuals. Similar changes in HIV-1 RNA load during zidovudine therapy were observed in p24 antigen-positive and -negative individuals. Analysis of the HIV-1 RNA response according to clinical outcome demonstrated that HIV-1 RNA copy numbers had declined significantly after 4 weeks of therapy in both non-progressors and progressors, but the decline in RNA load was much stronger in the non-progressors. HIV-1 RNA load in serum was shown to be useful for monitoring the response to antiviral therapy in p24 antigen-positive as well as negative individuals. Post-treatment changes in p24 antigen levels were not indicative for clinical outcome, whereas RNA copy numbers were. Therefore, it was concluded that monitoring p24 antigen levels was less valuable than HIV-1 RNA following antiretroviral therapy.

In Chapter 3, HIV-1 RNA levels as measured by two commercially available quantitative assays were compared. HIV-1 RNA levels were measured in stored serum samples from 24 Dutch zidovudine-treated participants of a zidovudine efficacy study (European-Australian Collaborative Group Study 017) at weeks -3, 0, 4 and 8, using quantitative nucleic acid sequence based

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amplification (NASBA; Organon Teknika) and quantitative reverse transcriptase-polymerase chain reaction (Amplicor; Roche Molecular Systems). HIV-1 RNA copy numbers and changes from baseline as measured by each assay were compared. Individual responses to treatment were compared using definitions based on the within-subject variation of each assay. Before treatment, HIV-1 RNA levels as measured by NASBA were 0.49 log₁₀ higher than the levels measured by the Amplicor assay (95% confidence interval (CI) 0.32-0.66). During treatment, this difference decreased significantly to 0.27 log₁₀ (95% CI 0.01-0.53; difference 0.22 log₁₀; 95% CI 0.05-0.37). The smaller difference between the results of the two assays during treatment was a consequence of a larger decline in RNA level as measured by NASBA compared with that measured by the Amplicor assay (mean change after 4 weeks 0.77 and 0.49 log₁₀, respectively). At week 8, the mean HIV-1 RNA level was still significantly below baseline values as measured by NASBA, but not when measured by the Amplicor assay. Discrepancies in individual responses as measured by the two assays were also observed. It was concluded that a marked difference existed between the NASBA and Amplicor quantitative assays, in both HIV-1 RNA copy numbers without treatment and changes in RNA level during treatment. These differences should be considered in interpreting analyses of clinical trials and relationships between HIV-1 RNA level and clinical outcome, as well as in the use of RNA level in the management of HIV infected patients.

Chapter 4 describes the evaluation of three procedures for the quantification of human immunodeficiency virus type 1 (HIV-1) RNA from plasma at three laboratories. The comparison involved the Quantiplex branched DNA assay (version 1.0) by Chiron Diagnostics, the NASBA-QT assay by Organon Teknika, and the Amplicor Monitor assay by Roche Molecular Systems. The laboratories performed each of the three assays with the same sets of reconstructed HIV-1-infected human plasma samples, cross-sectionally collected clinical plasma samples and longitudinally collected plasma samples from patients starting zidovudine therapy. Analysis of the reconstruction panel results for inter-laboratory variation demonstrated that no laboratory differences in results were detected for any of the assays. A comparison of the reproducibilities of duplicate samples analysed by batch and in separate assay runs demonstrated that the reproducibilities of the test results were similar within one assay and appeared to be independent of the HIV-1

concentration. The best reproducibility was obtained with the Quantiplex assay, but all three assays demonstrated equal reliability, which was independent of batched or unbatched analysis of replicate samples. Differences in the absolute concentrations calculated were observed for the assays, in particular in the analysis of reconstructed samples. In all assays, similar changes in plasma HIV-1 RNA concentrations were determined for longitudinally collected clinical samples.

The Delta trial, which was described in Chapter 5, was designed to test whether combinations of zidovudine with didanosine or zalcitabine were more effective than zidovudine alone in extending survival and delaying disease progression. This trial was randomised, double blind, and international. 3207 participants were allocated to either zidovudine (600 mg per day) alone (1055), zidovudine plus didanosine (400 mg per day) (1080), or zidovudine plus zalcitabine (2.25 mg per day) (1072). Participants either had symptoms of HIV disease (if AIDS, with a CD4 cell count of $> 50 \times 10^6/L$) or a CD4 count of less than $350 \times 10^6/L$; 2124 had not had zidovudine before (Delta 1) and 1083 had for at least 3 months (Delta 2). Over a median follow-up of 30 months, 699 participants died, and 936 of the 2765 without AIDS at entry developed AIDS or died. In participants who had not had zidovudine before, both combination regimens had substantial benefits in terms of survival (regardless of disease stage at entry); a relative reduction in mortality of 42%, compared to zidovudine alone (95% CI 25% to 55%), for zidovudine plus didanosine and of 32% (95% CI 22% to 47%) for zidovudine plus zalcitabine. In participants who had had zidovudine before, the addition of didanosine improved survival ($p=0.05$; relative reduction 23% [95% CI 0% to 41%]) but there was no direct evidence of benefit from the addition of zalcitabine ($p=0.47$; relative reduction 9% [95% CI 17% to 29%]). The overall difference in survival between the treatment groups was significant ($p<0.0001$; a relative reduction in mortality, compared to zidovudine alone, of 33% (95% CI 20% to 44%) for zidovudine plus didanosine and 21% (95% CI 6% to 34%) for zidovudine plus zalcitabine). Benefit in terms of disease progression was seen mainly in participants not previously treated with zidovudine and overall. There was no unexpected toxicity from the combination treatments. In conclusion, initiation of treatment with combinations of zidovudine plus didanosine or zalcitabine prolonged life and delayed disease progression compared with zidovudine

alone. The addition of didanosine to participants already treated with zidovudine also improved survival, although the benefit appeared to be less.

To explore the virological pathophysiology underlying the clinical findings of the Delta trial, changes in markers for viral load and antiretroviral-drug resistance during therapy were evaluated in three of the participating countries, France, the Netherlands, and the UK (Chapter 6). In total 240 zidovudine-naive HIV-1-infected patients who were randomly assigned zidovudine only (n=87), zidovudine plus didanosine (n=80), or zidovudine plus zalcitabine (n=73) were included. Viral load in peripheral-blood mononuclear cells and plasma was measured by quantitative culture. Plasma HIV-1 RNA was measured by reverse-transcriptase PCR amplification, and serum p24 antigen by ELISA. Resistance to antiretroviral drugs was measured phenotypically by culture and genotypically by detection and quantification of drug-related point mutations in the *pol* gene. Analyses were done by intention to treat. The reduction in viral load was greatest 4-12 weeks after the start of therapy and was most pronounced in the combination-therapy study groups (median reductions of RNA at 4 weeks 1.58, 1.28, and 0.49 log₁₀ copies/mL for zidovudine plus didanosine, zidovudine plus zalcitabine, and zidovudine only, respectively). RNA levels at 8 weeks were predictive of disease progression and death after allowance for baseline values.

At 48 weeks, the proportion of participants with phenotypic zidovudine resistance was similar in all three groups: didanosine and zalcitabine resistance were rare; zidovudine genomic resistance correlated with phenotypic resistance ($r=0.54$, $p<0.0001$) and developed earlier in the combined-therapy groups. However, participants in the zidovudine monotherapy group had higher circulating loads of resistant virus than those in the combined-therapy groups. We concluded that combined antiretroviral therapy was more efficient at lowering virus load than monotherapy. Although zidovudine resistance was common in monotherapy and combined-therapy groups, circulating concentrations of resistant virus were substantially lower in the combination groups, which was likely a result of the continued antiviral activity of didanosine or zalcitabine.

A substantial number of patients with advanced HIV infection suffer from intractable diarrhoea. In Chapter 7 we evaluated whether potent antiretroviral therapy could alleviate such diarrhoea in an open randomised study. The effect of the HIV protease inhibitor indinavir in combination with

nucleoside analogue reverse transcriptase inhibitors on chronic HIV-related diarrhoea was investigated in 14 late-stage ($CD4^+$ lymphocyte count $\leq 50 \times 10^6$ cells/L) HIV-infected patients. Data concerning stool frequency, stool consistency and antidiarrhoeal drug use were collected in daily diaries over a 24-week period. Endpoints of the study were reduction of stool frequency, improvement of stool consistency, weight gain, and in case of diarrhoea due to *Enterocytozoon bienewisi* or *Cryptosporidium* sp. disappearance of these parasites from stool. Thirteen patients started the study drug indinavir. One patient died after 1 week and one patient withdrew prematurely after 18 weeks. Median stool frequency declined from 5.8 daily at baseline to 2.3 daily after 24 weeks ($p=0.04$). Stool consistency improved considerably over the study period: before treatment 56% of stools were watery and 0% were formed; at week 24 these figures were 0 and 35%, respectively. Body weight increased significantly with a median increment of 6.6 kg at week 24 ($p=0.0006$). In two out of six patients with microsporidiosis and both patients with cryptosporidiosis, stools were free of parasites at week 24. Five out of six patients who used non-specific antidiarrhoeal medication on a regular basis prior to the study had ceased to do so at the end. Consequently, the use of potent antiretroviral therapy in patients with advanced HIV infection can improve chronic HIV-related diarrhoea and in some cases lead to disappearance of *E. bienewisi* and *Cryptosporidium* sp. from the stools.

The widespread use of this highly active antiretroviral therapy (HAART) has improved rates of $CD4$ lymphocyte recovery and decreased the incidence of HIV-1-related morbidity and mortality. In Chapter 8 it was assessed whether prophylaxis against *Pneumocystis carinii* pneumonia (PCP) can be safely discontinued after HAART is started. A total of 7333 HIV-1-infected patients were investigated already enrolled in EuroSIDA, a continuing prospective observational cohort study in 52 centres across Europe and Israel. Person-years analysis was performed of the rate of discontinuation of PCP prophylaxis and of the incidence of PCP after the introduction of HAART into clinical practice from July, 1996. The rate of discontinuation of primary and secondary PCP prophylaxis increased up to 21.9 discontinuations per 100 person-years of follow-up after March, 1998. 378 patients discontinued primary (319) or secondary (59) prophylaxis a median of 10 months after starting HAART. At discontinuation for primary and secondary prophylaxis, respectively, the median $CD4$ lymphocyte counts were 274 cells/ μ L and 270

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cells/ μL , the median plasma HIV-1 RNA load 500 copies/mL, and the median lowest recorded CD4 lymphocyte counts 123 cells/ μL and 60 cells/ μL . During 247 person-years of follow-up, no patient developed PCP (incidence density 0 [95% CI 0-1.5]). Therefore, the risk of PCP after stopping primary prophylaxis, especially in patients on HAART with a rise in CD4 lymphocyte count to more than 200 cells/ μL , is sufficiently low to warrant discontinuation of primary PCP prophylaxis.

A comparison of viral suppression using three drugs or five drugs is described in Chapter 9. Two open-label studies using a three-drug (zidovudine, lamivudine and ritonavir) and a five-drug regimen (zidovudine, lamivudine, abacavir, indinavir and nevirapine) in study-drug-naïve patients, except for one in the five-drug study. Participants with $\geq 10\,000$ HIV-1 RNA copies/mL in plasma at baseline were compared by means of Kaplan-Meier curves for time to < 50 copies/mL, as well as linear regression analysis for the first phase of decline using log-transformed copy numbers. The elimination rate constants for HIV-1 RNA in 15 participants of the three-drug study were compared with nine participants of the five-drug study. The level of < 50 copies/mL was reached earlier when using the five-drug than when using the three-drug regimen (p log rank = 0.0005): median time to reach this level was 4 weeks and 12 weeks, respectively. No differences were found in HIV-1 RNA elimination rate constants in the first 2 weeks after the initiation of therapy. When the viral load declines were calculated from day 2 onwards, adjusting for differences in pharmacological delay of the drugs used, again no differences in early viral load decline were found between the two regimens. With the five drugs used in this study, the median time to reach < 50 HIV-1 RNA copies/mL was 8 weeks shorter than with the three-drug regimen. This finding showed that suppression of viral load in HIV-infection by standard triple-drug therapy can be improved upon.

In general, randomisation in clinical trials assessing the efficacy of a medical intervention is the methodological gold standard. Blocking and stratification are used in random treatment assignments to preserve treatment balance with respect to important covariates. However, these technologies are associated with considerable increase of predictability of treatment allocations when used in open intervention studies. Especially in small studies it is of importance to maintain balance without increased predictability of treatment allocations. Clinical trials on antiretroviral treatment in HIV infection are

becoming short running, small sample sized open-intervention trials with in most instances a number of known prognostic factors like the number of CD4⁺ T-lymphocytes, HIV-1 RNA copy number, and the use of antiviral treatment before study participation. In Chapter 10 it is demonstrated that minimisation technology reduced imbalance in treatment allocation, although with an increase of predictability. In this chapter we developed a minimisation technology in which the assignment probability is a function of the degree of imbalance. To investigate advantages in balance and predictability of this technique, we compared different randomisation technologies ranging from complete randomisation to maximal balancing. Using the degree of imbalance as a function for the probability of treatment allocation will reduce the allocation predictability without the loss of balance.

