THE USE OF AMINOGLYCOSIDE ANTIBIOTIC THERAPY IN NEUTROPAENIC PATIENTS WITH HAEMATOLOGICAL DISEASE.

Dissertation submitted for completion of requirements for the MMed degree in the Department of Medicine at the University of Cape Town by Clive Steven Zent MBBCh(Rand), FCP(SA) in January 1991.

This work is dedicated to my wife Janet whose help and support made it possible.

I, Clive Steven Zent hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signed by candidate

17 January 1991

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THE USE OF AMINOGLYCOSIDES IN THE THERAPY OF FEBRILE NEUTROPAENIC PATIENTS WITH HAEMATOLOGICAL DISEASE.

ABSTRACT

The use of aminoglycosides in the treatment of the febrile neutropaenic patient with haematological disease is difficult and often suboptimal. This study reviews the available literature to establish therapeutic guidelines in this population and then reports the use of a Bayesian statistics based predictive model to implement and manage therapy in 10 patients.

A review of the literature on aminoglycoside pharmacology and clinical use is essential to determine therapeutic guidelines for this population. Aminoglycosides are amino sugars in glycosidic linkage and are polycations at physiological pH. The antibiotic effect is mediated through inhibition of protein synthesis and disruption of cell membrane integrity. Principal use is in treatment of Gram negative infection although aminoglycosides have activity against some Gram positive organisms including staphylococci. Aminoglycosides are inactive against anaerobes. Acquired resistance is mediated by bacterial enzymatic drug metabolism. Aminoglycosides are nephro- and ototoxic, this is the major constraint in clinical use.

The polar nature of the aminoglycosides results in restriction of drug distribution to the extracellular fluid space with clearance by glomerular filtration. Although simple and predictable pharmacokinetics may be expected, this is not observed in practice. Kinetic parameters correlate poorly with both physiological variables and disease states. There is also evidence that volume of distribution (Vd) is increased significantly in seriously ill patients and those with neutropaenia.

The toxic/therapeutic ratio for aminoglycosides is low. Planning of

optimal therapy requires determination of the maximum drug level and dosage frequency that will not cause toxicity. This demands a thorough understanding of the relationship between drug kinetics and dynamics. Aminoglycoside antibiotic effect in susceptible organisms is depends on the drug level achieved in the bacterial cytoplasm. Clinical studies have established that the early achievement of high peak levels and high maximum peak levels are the best predictors of therapeutic response. In vitro and animal studies suggest that the peak level:minimal inhibitory concentration (MIC) ratio and antibiotic killing rates are the best predictors of antibiotic effect. The post antibiotic effect (PAE) is well established for aminoglycosides and has been investigated extensively as the basis for once daily dose therapy. However this is not currently an option for neutropaenic patients.

Aminoglycoside ototoxicity correlates with the area under the concentration-time curve (AUC), cumulative dose, duration of therapy and trough drug levels. The trough level is the only variable that can be monitored and influenced during therapy.

Nephrotoxicity also correlates with AUC, cumulative dose and duration of therapy but the relationship with measured drug levels is less well established.

The development of rapid, cheap and reliable assay methods for aminoglycosides has allowed the evolution of therapeutic drug monitoring (TDM). Early experience with empiric and nomogram based methods was disappointing and has resulted in the development of computer based individualized predictive models with significantly improved performance. The method developed by Sawchuck and Zaske (SZ) and the Bayesian statistical based methods are both well validated for general hospital patients and intensive care unit (ICU) patients. The SZ method has also been used in neutropaenic patient with haematological disease but there is no published report

of use of the Bayesian method in this population.

Empiric antibiotic therapy in febrile neutropaenic patients has resulted in a dramatic improval in survival. Gram negative organisms and staphylococci comprise the majority of documented infections in this population. Antibiotic protocols usually comprise combinations of a Beta-lactam (B-lactam) and aminoglycoside although single and double B-lactam regimens are also used. A review of reports of treatment efficacy and toxicity as well as resistance patterns, suggests that aminoglycoside containing regimens are still optimal therapy in this population.

The available evidence thus suggests that early achievement of therapeutic peak levels (30 +/- 10 mg/l) with avoidance of toxic trough levels (>6 mg/l) using multiple daily intravenous (IV) injections results in optimal amikacin therapy.

Routine therapeutic drug monitoring for aminoglycosides has not yet been used at Groote Schuur Hospital. The purpose of this study was to validate a Bayesian method in a population at high risk of treatment failure and aminoglycoside toxicity in whom use of this method has not previously been reported.

Sequential admissions to the Department of Haematology Isolation Unit at Groote Schuur Hospital/University of Cape Town who had haematological disorders, required aminoglycoside therapy according to Unit treatment protocol criteria and were neutropaenic during therapy were enroled. Treatment was initiated by Unit medical staff and blood specimens obtained for aminoglycoside level assay by nursing staff at 1 hour after the start of administration with one further level being obtained in the first dose interval.

Assays were performed using the flourescent antibody method (Abbott TDX). Patient physiological parameters and drug levels were entered into the Bayesian model (OPT Clydesoft) and initial kinetic parameter and dose estimations derived. These were implemented

within 24 hours of the onset of therapy. Peak and trough levels were subsequently assayed at least twice a week and kinetics and dose estimations re calculated. Therapy was revised according to derived recommendations. Prediction accuracy was determined by comparison of achieved and predicted levels. Bias was measured by calculating mean error of prediction (ME) and precision by the calculation of mean absolute error (MAE) and root mean squared error (RMSE). The percentage of achieved therapeutic levels was also calculated.

Ten sequential Unit admissions satisfying enrolment criteria were studied. Patient age ranged from 15 to 60 years with 6 males and 4 females. There was one patient death during the study period. Nine patients received chemotherapy and the three bone marrow transplant patients also received high dose radiotherapy. An initial 1 hour peak level was obtained in all patients but the second initial level was obtained in only 9 patients with 1 patients initial estimates requiring use of levels from the second dosage interval. 38 batches of assays were performed and the median control value of 14.7 +/- 0.5 mg/l was within the manufacturers accuracy and variability specifications.

Prediction performance was assessed using 51 paired predicted versus achieved data sets. Overall peak level prediction was found to be both unbiased and precise while predictions for trough levels were biased and precise. Predictions based on first dose level measurements were unbiased but imprecise for peak levels and biased but precise for trough levels. There was an overall achievement of therapeutic peak levels for 80% and therapeutic trough levels for 94% of levels. 7 patients achieved therapeutic peak levels using first dose predictions. In 5 patients all measured levels were in the therapeutic range.

Patient estimated mean kinetic parameters fell within the range of

standard deviation of reported values for amikacin and could thus not be considered significantly different.

The use of the OPT Bayesian program resulted in acceptable but suboptimal dosage predictions in this study. The result was inferior to that achieved in other reported studies and especially those using initial population kinetics derived from an equivalent sample of the same population. The failure to find a significant difference in kinetic parameters might have been a result of small sample size or inaccuracy of the Bayesian method in calculation of exact kinetic parameters or may represent a true result. An attempt was thus made to alter the OPT initial population data to improve prediction accuracy. This was not successful. The contribution of experimental method error influencing the prediction performance is unlikely to be significant. A major limitation to the performance of any predictive model remains the inability to predict inter and intrapatient kinetic variability.

This study concludes that aminoglycosides remain essential antibiotics in the therapy of neutropaenic patients with haematological disease. Optimal therapy requires multidose daily dosing with early and sustained achievement of therapeutic peak and non toxic trough levels. A literature review suggests that this goal is only attainable with the use of a TDM program such as the OPT model. Use of the OPT model for patients at GSH resulted in the achievement of acceptable but suboptimal prediction accuracy. This suggests that a Bayesian TDM system can be used in this population although improvement of accuracy is desirable. Model performance can be improved with increased experience and use of more appropriate initial kinetic parameters. However the basic constraints imposed by aminoglycoside kinetic variability will continue to limit performance accuracy until a better understanding of the mechanism and correlates of this variability is developed.

1. INTRODUCTION

Aminoglycosides are valuable antibiotics with a well established role in clinical medicine.

Therapeutic use is limited by the need to maximize the concentration dependent antibiotic efficacy of a drug with a low therapeutic:toxic ratio. Accurate drug use is thus essential for effective and safe treatment. Achievement of this goal is complicated by the lack of sufficient data to establish reliable target drug level and dosage frequency guidelines. This is a result of the difficulty in performing controlled trials of effectiveness in seriously ill patients in whom physiological and disease variables cannot be adequately controlled. The complexity of therapy is compounded by the large inter- and intrapatient pharmacokinetic variability for aminoglycosides especially in the seriously ill. Treatment of neutropaenic patients requires further consideration due to reports of altered kinetics and especially volume of distribution (Vd) in this population.

This study will review the pharmacology of aminoglycosides so as to explain the basis of current treatment regimens. Clinical medicine requires therapeutic guidelines for the use of aminoglycosides in neutropaenic patients with haematological disease despite the uncertain and incomplete nature of available data. Provisional treatment recommendations are thus proposed.

The need for therapeutic drug monitoring (TDM) for accurate implementation of aminoglycoside treatment in the seriously ill patient is well established. This evidence is reviewed. The novel experimental use of a Bayesian TDM pharmacokinetic model for amikacin therapy in neutropaenic patients with haematological disease in a study performed in the Department of Haematology Source Isolation Unit at Groote Schuur Hospital is then reported.

1.1 AMINOGLYCOSIDE CHEMISTRY

The aminoglycoside antibiotics are amino sugars in glycosidic linkage (aminoglycosidic aminocyclitols). The aminoglycosides commonly used in South Africa for systemic therapy (gentamicin, tobramycin, amikacin and netilmicin) have similar chemical and pharmacological characteristics.

The agent used in this study is amikacin which was first synthesized in 1972. It is a semisynthetic antibiotic derived from kanamycin by acylation of the 1 amino group of the 2 deoxystreptamine moiety with 2 hydroxy 4 amino butyric acid (1).

1.2 PHARMACODYNAMICS

1.2.1 DRUG ACTION

The aminoglycosides are rapidly bactericidal in adequate concentration for susceptible organisms. The mechanism of action is not fully understood and is believed to involve several steps. Research has been conducted mainly on Gram negative organisms. Aminoglycosides enter the periplasmic space through aqueous channels formed by porin protein (1). Transport across the inner or cytoplasmic membrane is an energy dependant active process which is inhibited by divalent cations eg Ca++ and Mg++ (2), increased osmolality, decreased pH and an anaerobic milieu (1)(3). During this phase the drug probably causes disruption of the cytoplasmic membrane, initially causing leakage of small ions and later larger molecules. This is believed to be a major component of antibiotic action (1).

Within the bacterial cytoplasm, aminoglycosides bind to ribosomal components with greater affinity for 30S than 50S subunits (1). This results in both the inhibition of protein synthesis and the

accumulation of abnormal initiation complexes. There is loss of translation fidelity with misreading of the mRNA code (1).
A combination of disruption of the integrity of the inner or cytoplasmic membrane integrity and inhibition and loss of fidelity of protein synthesis may explain the rapidly lethal effect of the drug.

1.2.2. DRUG RESISTANCE

Clinically relevant resistance to the action of aminoglycosides may be due to chemical drug inactivation, impaired penetration of the bacterial cell membrane or enzymatic degradation in the periplasmic space.
Chemical inactivation occurs in areas of low pH eg pus, and high divalent cation concentration and osmolality eg the renal medulla.
Failure of permeation of the cytoplasmic membrane occurs in anaerobic organisms which have a poorly developed active transport mechanism (1).
The principal mechanism of acquired resistance is the enzymatic inactivation of the drug in the periplasmic space. Frequency of such resistance is largely determined by the extent and duration of antibiotic use (4). Aminoglycosides are inactivated by adenylation, phosphorylation and/or acetylation of specific hydroxyl or amino groups (1)(4) by enzymes coded by plasmids and resistance transfer factors (1). Several resistance enzymes may coexist in the same organism and sequential enzyme action is important as a mechanism for amikacin resistance (4).

1.3 PHARMACOKINETICS

The kinetics of aminoglycosides are determined by their chemical characteristics. Aminoglycosides are polycations at physiological pH and therefore cannot easily cross biological membranes and have limited access to body fluid spaces.

1.3.1 ADMINISTRATION

Aminoglycoside absorption from the gastrointestinal tract is < 1% (1). Parenteral administration is thus essential for system use. Intravenous injection is a reliable method and is generally employed in ill patients with unpredictable tissue perfusion. Intramuscular injection yields peak levels equivalent to those obtained with intravenous infusion. However absorption from muscle is unreliable in the elderly and seriously ill.

The most popular method of administration is pulsed continuous intravenous infusion which is believed to be safe and effective (3). Bolus injection may result in increased ototoxicity (3), and continuous infusion is of no proven therapeutic value and has increased toxic potential (3).

1.3.2. DISTRIBUTION

Aminoglyosides are charged at physiological pH and distribution is restricted to the extra cellular space. Penetration of extravascular fluid spaces is variable. There is good penetration of cochlear and vestibular perilymph and endolymph (1) and concentrations 5 -10 x those of serum are achieved in the renal tissue fluid (1)(5). Entry of drug into the pleura, ascitic and synovial fluid is slow but high levels are obtained with continued use (1)(3). Penetration of bronchial secretions is variable (1).

There is active hepatic secretion into bile with levels of approximately 30% of that of serum being achieved (1). Penetration of prostatic and amniotic fluid is slow and incomplete (3). There is poor penetration of the blood brain barrier. Cerebrospinal spinal fluid levels reach only 20% of the serum level in the presence of meningeal inflammation (1). Aminoglyosides do not enter the eye (1). The drug is excluded from all cells with the exception of those of the proximal renal tubules where uptake is by pinoctosis (6). Aminoglyoside plasma protein binding is less than 10% and has no kinetic significance (1).

1.3.3. ELIMINATION

90% of excretion is by renal glomerular filtration with a small amount of drug being excreted in the bile (3). A small but variable degree of renal tubular reabsorption occurs. Aminoglycoside clearance (Cl) is approximately 65% that of creatinine clearance (1)(7) but correlation is variable as creatinine is excreted by glomerular filtration and tubular secretion (7)(8). A wide variation of drug clearance occurs in both health and disease (3).

1.3.4. KINETIC MODELS

Aminoglyosides are restricted to the extracellular fluid, have minimal plasma protein binding and are cleared primarily by glomerular filtration . Kinetics should thus be expected to be simple. However this is not observed in practise and there is no reliable and accurate kinetic model explaining observed experimental concentration - time curves.

Aminoglyosides have at least triexponential kinetics (9) and the optimal model for accurate fit comprises three compartments. The first component represents the rapid distribution phase during drug administration and has a half life (t1/2) of less than one hour.

The second compartment is the central compartment of distribution and has a volume of distribution (Vd) of 0.11 to 0.31 l/kg body weight and a t1/2 of approximately two hours which corresponds closely to the elimination t1/2 reported in the one compartment model analysis. The third compartment represents deep tissue accumulation of the drug. This occurs principally in renal tissue, which has the highest tissue to serum concentration ratio, and skeletal muscle which has the greatest storage capacity. The Vd of this compartment is 0.45 to 1.34 l/kg with a t1/2 ranging from 40 hours to several days (3)(10).
Use of the three compartment model requires multiple samples to establish an accurate concentration time curve and the use of a complex mathematical model to determine kinetic parameters.

A simplified one compartment model has been shown to be sufficiently accurate for clinically valuable estimation of aminoglycoside kinetic parameters (3). Use of this model requires IV drug administration with sampling delayed until the completion of the initial distribution phase thus allowing the first compartment to be ignored for subsequent modelling purposes (11)(12). There is no simple method of compensating for the inaccuracy caused by the third or deep tissue compartment. Gradual accumulation in the slow exchange deep muscle and renal compartment results in some inaccuracy when using the one compartment model which fails to predict the slow rise in peak and trough levels with prolonged use of the drug especially in excess of 10 days (10)(13).

1.3.5. VARIATION IN KINETIC PARAMETERS

Aminoglyoside kinetic parameters demonstrate considerable inter- and intra individual variability due to both physiological and pathological factors. This variability remains poorly defined and

explained. However a discussion of the available data is essential to facilitate an understanding of the intrinsic limitations of kinetic predictions in therapeutics.
The initial kinetic research on aminoglyoside was performed on healthy subjects and usually involved studies of single dose administration. Subsequent kinetic studies in clinical practise have revealed significantly different parameters with the most prominent feature being wide variations in Vd, Cl and t 1/2.

i) Physiological Factors

a) Age: Paediatric Vd is significantly greater and t1/2 significantly lower than adults, but no correlation exists between Vd and age if corrected for creatinine clearance. (3)(14).
b) Body Weight: Extra cellular fluid (ECF) volume usually correlates with body weight. The Vd of aminoglycosides has been estimated at 19% of ideal body weight plus 6% of weight in excess of ideal body weight (3). However some studies have shown body weight, both actual and ideal, to correlate poorly with measured Vd , a study performed on patients in a Surgical Intensive Care Unit (ICU) yielded a r value of 0.32 for the relationship between Vd and body weight (12). Most patients have a Vd for aminoglycosides in excess of the value for the volume of extra cellular fluid calculated from body weight (12).
c) Sex: Females tend to have a lower Vd because of decreased muscle mass and a decreased ECF: weight ratio . Pregnancy causes both an increase in Vd as the ECF compartment increases and an increase in Cl with increased cardiac output and GFR (3).
d) Renal Function: There is no reliable clinically available indicator of the renal clearance rate of aminoglycosides. A wide variation in elimination occurs amongst patients with normal creatinine levels and creatinine clearance rates. In healthy

volunteers the t1/2 for amikacin ranges from 0.8 to 2.8 hours and for gentamicin from 2.5 to 4 hours (3). The relationship between gentamicin clearance and calculated creatinine clearance is r = 0.42 (7) and r = 0.55 for correlation with measured creatinine clearance (15). The r value for correlation between t1/2 and serum creatinine has been reported as 0.35 (11). The variability in drug elimination rate as predicted by changes in serum creatinine has been reported as 50% for Surgical ICU patients (12) and as between 40% and 50% in other studies (3)(16). Thus although renal function is the most important predictor of clearance, the correlation between creatinine clearance and aminoglycoside is relatively poor.

e) Temperature: Experimental work in dogs using endotoxin has demonstrated an increase in Cl during pyrexial illnesses secondary to an increase in GFR (3).

Age, weight, sex, renal function and temperature are physiological variables affecting kinetic parameters. However, correlation is relatively poor and resultant kinetic parameters predictions unreliable.

ii) Effects of Disease

Aminoglyosides kinetics show increased inter- and intrapatient variability in disease states. This can only be partially explained by the alterations in fluid status and renal function which are especially prevalent in patients with septicaemia.

a) Infection: Significant increases in Vd occur except when patients are severely dehydrated (15)(17). A wide range in parameters occurs in gram negative pneumonia with a Vd mean of 0.21 l/kg with a range of 0.10 - 0.46 l/kg and a t1/2 of 0.7 - 6.6 hours being reported in patients with normal serum creatinine levels (18). Intrapatient variations in Vd over 24 hours range from

decreases of 42% to increases of 91% with a mean absolute change of 20% (11). Intrapatient changes in clearance range from a reduction of 39% to an increase of 36% with a mean absolute change of 17% (11). A large study of critically ill surgical patients (19) revealed major intrapatient variations during the course of therapy. The Vd changed by more than 1 litre in 83% of patients and by more than 5 litres in 48% of patients with a change in clearance of greater than 10 ml/min in 51% of patients with the highest value being 108 ml/min. Poor and inconsistent correlation between changes in body weight and Vd and changes in creatinine clearance and aminoglycoside clearance was found. There is no adequate explanation for these findings although some authors propose that septic patients may initially have large volumes of distribution which decrease as the patients clinical condition improves (11). The increased measured Vd may also be due to failure to consider exogenous volumes administered as well as increased microvascular permeability with loss of fluid into the interstitial space resulting in ECF accumulation (12).

b) ECF volume alteration: Ascites, congestive cardiac failure and the presence of pleural effusions increase Vd (3)(15).

c) Burns: Significant increases in both Cl and Vd occur (3)(20).

d) Cystic fibrosis: Increased drug clearance has been documented (3).

e) The neutropaenic patient: Several studies have suggested significant differences in aminoglycoside kinetics in neutropaenic patients. However this issue remains controversial.

In a prospective uncontrolled study on 35 patients (21) aminoglycoside Vd and Cl were found to be significantly increased in patients with solid and haematological malignancy treated with empiric antibiotics. There was no correlation between kinetic changes and sex, type of malignancy, age or serum albumin.

Clearance was increased 63% (P< 0.05) and Vd increased 14% (P < 0.05) as compared to population kinetic parameters used. Due to the use of a historical control and the known variability in population parameters, the value of this study in establishing a relationship between changes in kinetic parameters and white blood cell counts is small.

Bianco et al report a prospective control comparison of gentamicin kinetics in 34 febrile neutropaenic patients as compared to 40 non neutropaenic patients matched for age, sex , weight and renal function (7). The study was predicted to have a 95% chance of predicting a 22.5% difference in Vd. No difference in kinetic parameters was found but both groups had a volume of distribution mean of 0.31 l/kg actual body weight (ABW) which is higher than the 0.25% l/kg AWB used as a control in the previous study.

Andrejak and Hary compared kinetics in eight non febrile severely neutropaenic patients (count less than a 500/mm^3) with eight healthy volunteers using amikacin and a two compartment model with nine post infusion measurements (22). They conclude that both Cl and Vd are significantly increased in the neutropenic patients. The mean +/- SD value for Vd was 0.45 +/- 0.05 l/kg ABW and the clearance mean +/- SD was 2.55 +/- 0.19 ml/min/kg. Despite the small numbers, the study used accurate kinetic methods and excluded the effect of pyrexia. The authors propose that neutropenia is associated with vascular endothelial damage which causes interstitial oedema thus increasing both the extra cellular fluid volume and the volume of the distribution of aminoglycosides.

Zeitany et al conducted a prospective study on aminoglycoside parameters in 27 patients with haematological malignancy, fever and neutropaenia (23) . A matched control group of eighteen patients was used. The authors conclude that Vd is consistently and significantly increased in haematological malignancy with a mean +/-

standard deviation (SD) value of 0.40 +/-0.10 l/kg compared to 0.27 +/- 0.05 l/kg for the control group (p < 0.004). No difference between diagnostic groups was noted. Clearance was significantly increased at a mean +/- SD value of 116.7 +/- 48.9 ml/min compared to 68.6 +/-26.7 ml/min for the control group (P< 0.005). Patients in this study required larger amounts of drug to achieve therapeutic levels with a mean increase in requirement exceeding the manufacturers recommendations by 100%. No significant difference was reported for kinetic parameters for patients before and after treatment with chemotherapy.

Manny and Hutson report a retrospective study in 32 haematology and oncology patients with no clinically evident extracellular fluid accumulations (24). The authors did not comment on neutrophil counts or treatment status with respect to the administration of chemotherapy. The Vd was reported as mean +/-SD of 0.41 +/-0.13 l/kg.

Phillips et al (25) reported on Vd for gentamicin in 24 patients with haematological disease and neutropaenic fever. An increased Vd was found in the study population compared to a literature derived value for the "general population" i.e. mean +/-SD of 0.425 +/- 0.041 l/kg compared to 0.249 +/- 0.006 l/kg (P<0.001). No patients had pleural or peritoneal effusions. No significant correlation was found between Vd and age, sex, serum albumin level, haematocrit, platelet count, neutrophil count or disease type or activity. Chemotherapy in the preceding 21 days was associated with an increased Vd.

The currently available data suggests that Vd is increased for aminoglycosides in the neutropaenic patient. However no prospective controlled study of kinetics in this population has been reported and the issue remains controversial. There is also no tenable

mechanism to support a claim for altered kinetics. The significance of neutropaenia in the therapeutic use of aminoglycosides and its role in the use of TDM remain to be established.

1.4 The KINETIC - DYNAMIC RELATIONSHIP

Determining the relationship between aminoglycoside kinetic parameters and therapeutic effects, facilitates safe and effective drug use. This section discusses the relationship between the concentration - time curve and aminoglycoside antibiotic and toxic effects so as to propose a rational basis for therapeutic use.

1.4.1. THE ANTIBIOTIC EFFECT OF AMINOGLYCOSIDES

Maximal antibiotic effect occurs when high tissue are achieved and maintained. This may however occurs at the expense of unacceptable toxicity. Thus an optimal treatment regimen based on reliable data on effective drug levels and using the lowest dose for the shortest period possible should be determined.

Current knowledge is based on in vitro and animal experiments and an incomplete reported clinical experience.

i) Drug Administration

Use of the intravenous (IV) route is essential for reliable delivery to seriously ill patients with intramuscular (IM) administration being reserved for less serious infection in younger patients with well preserved muscle mass.

Continuous IV infusion has been used (26). This is probably less effective and more toxic and expensive than intermittent dosing (27). Interval therapy can be either by intermittent infusion or bolus dose. The bolus dose method is cheaper and easier but believed by several authorities to have increased toxic potential without therapeutic advantage (3)(28). Intermittent infusion at a constant rate over a period ranging from 15 to 60 minutes is

currently the most popular method. This decreases the height of the peak level during the initial distribution phase and together with a delay in initial sampling facilitates use of a single compartment kinetic model for prediction.

This study uses a constant 30 minute infusion with peak level sampling at 1 hour after the start of the infusion.

ii) Setting Target Kinetic Parameters

Optimal peak and trough target levels and dosing frequency are not known. The following discussion reviews the kinetic -dynamic and microbiological data relevant to establishing acceptable therapeutic guidelines.

Aminoglycoside bactericidal effect is principally dose dependent (29). Efficacy depends on the antibiotic levels achieved within the bacterium. Drug entry occurs down a concentration gradient although a refractory period has been demonstrated after about 6 hours of continuous exposure (30). There is no evidence that saturation of antibiotic effect occurs with increasing concentration (31). The time of exposure to high levels of drug may be of lesser importance with variable correlation with bacteriocidal effect among different organisms. Pseudomonas and Serratia species appear to require longer periods of drug exposure to ensure organism death (32). Kill rates have been shown to correlate with the area under the concentration - time curve (29). Further considerations in planning treatment are the therapeutic value of the post antibiotic effect, the importance of rapid achievement of therapeutic levels after commencement of therapy and the risk of bacterial regrowth during prolonged periods of suboptimal antibiotic levels.

a) Clinical Studies: Several studies attempting to identify kinetic parameters determining drug efficacy are discussed. It is important to note these studies are uncontrolled, it is considered unethical to fail to treat Gram negative infection. However historical

controls from the pre-antibiotic era can be used for approximate comparison. Prior to the use of antibiotics documented Gram negative bacterial infection was frequently but not universally fatal with a survival of up to 68% being reported (10).

In a metaanalysis of 4 randomised double-blind studies comparing the use of various aminoglycoside containing regimens in Gram negative infections (33) Moore et al found that factors relating to one hour peak drug levels were significant in predicting the therapeutic response. Early achievement ie. within 24 to 48 hours, of therapeutic peak levels (> 20 mg/l for amikacin) was a significant factor improving therapeutic response and survival.

The individual report of one of the studies analysed by Moore et al in (33) correlates peak aminoglycoside levels with therapeutic outcome in 37 patients with Gram-negative pneumonia (34). Peak levels (> 24 mg/l for amikacin) were associated with improved survival. This was shown to be a more significant factor than initial temperature, polymorph count or age. The achievement of a maximum peak level during therapy of > 28 mg/l was an independent significant correlate for survival.

A further study by Moore et al (35) reported the correlation of maximum and mean peak concentration / minimal inhibitory concentration (MIC) with clinical response. A maximum peak level / MIC of > 4 correlated with a response rate of 70% which increased to 80-90% if the ratio was greater than 1:8 (36). There was no correlation between trough level or geometric mean of the serum concentration and clinical outcome. This finding is supported by the results of other studies (23) (33).

b) Clinico - Microbiological Studies: Attempts have been made to define therapeutic levels on the basis of in vitro bacterial studies and measurement of the correlation between drug levels, serum bacteriocidal characteristics and clinical response. This approach

provides valuable clinical data but does suffer from several distinct limitations:

1) Tissue fluid aminoglycoside levels correlate with serum levels (5). However the relationship between drug dose and serum levels is inconsistent with drug levels changing constantly during the dosage interval. Tissue accumulation of aminoglycosides also occurs with ongoing use. Thus neither knowledge of the size of drug dose used nor of serum drug levels achieved reliably predict tissue drug levels. The clinical value of the observed in vitro response to a constant drug level is thus limited.

2) Interpretation of the significance of in vitro antibiotic effectiveness testing is difficult. Early antibiotic research using penicillin in the 1940's established the importance of the serum concentration :MIC ratio in measuring antibiotic effects (10). Due to the antibiotic characteristics of beta lactams (B-lactams), the principal of requiring the maintenance of drug levels above the MIC for that agent for most of the drug interval was established. This is however neither necessarily true or optimal for aminoglycosides which have fundamentally different antibiotic effects and kinetic -dynamic relationships. Furthermore testing of the MIC has significant limitations. While MIC is valuable for determining bacterial resistance to an antibiotic it is of less use in quantitating bacterial susceptibility to a drug (32). This is primarily due to the larger in vivo bacterial load (10) and the inability of the MIC to take into account the dynamic nature of the inter dose drug level. The antibiotic killing rate has thus been proposed as a more accurate measure of antibiotic effectiveness (32). This measures the time kill and regrowth patterns of cultures exposed to patient serum.

3) Uncertainty as to the microbiological mechanism and clinical significance of the post antibiotic effect (PAE). The PAE is the

observed in vitro phenomenon of persistent suppression of bacterial growth after cessation of exposure to antibiotic. It is defined as the difference in time taken for previously drug exposed cultures to increase ten fold above the count prior to drug removal as compared to untreated cultures (29). The duration of aminoglycoside PAE increases with higher drug concentrations and after longer periods of exposure (27)(29).

Current data suggests that aminoglycoside antimicrobial activity can best be described by a combining data for MIC and minimal bacteriocidal concentration (MBC), the rate of bacterial killing, and the sub MIC and post antibiotic effects. The antibiotic killing rate and PAE data for aminoglycosides will be discussed in further detail.

The antibiotic killing rate is a dynamic measure of antibiotic efficacy that is performed on serum levels taken at timed intervals after a dose. A study using serum collected from healthy volunteers after a single dose of amikacin has been reported (32). Serum killing rates were determined for standard strains of E. coli, Enterobacter cloacae, Serratia marcescens and Pseudomonas aeroginosa using samples taken after a 30 minute infusion of either 7.5 mg/kg or 15 mg/kg amikacin IV. A one hour peak level of 20 - 30 mg/l was achieved at the lower dose which had substantial but submaximal bacteriocidal activity against E. coli and Enterobacter cloacae while the 15mg/kg dose achieved a one hour peak level ranging from 45 - 77 mg/l which was fully bacteriocidal for these organisms. The minimum concentration of regrowth (MCR) for these organisms was reached at 3 hours for the 7.5 mg/kg and 6 hours for the 15 mg/kg doses. The results for Pseudomonas aeroginosa and Serratia marcescens show relatively decreased bacteriocidal activity allowing determination of an index of surviving bacteria. This was

significantly less for the larger dose at 3 hours but the percentage for both doses ranges from 50 - 75%.

The PAE for aminoglycosides is only significant if organisms are exposed to levels exceeding 2 x MIC for periods of at least 2 hours (27) with maximal effect reported at 5 - 10 x MIC for a minimum exposure period of 2 hours (16)(31). The mechanism of PAE is unknown. Proposed mechanisms are persistence of the drug at the site of action and drug induced non lethal damage (27). A wide range of PAE durations have been reported. Aminoglycoside PAE against various organisms has been reported as 0.5 - 1.5 hr for Staphlococcus aureus, 0.5 -2.5 hr against Enterobacteriacae and 1.5 - 2.5 hr against Pseudomonas aeroginosa (29). Other publications quote a non specific period of 2 - 6 hr (27)(31).

c) The Neutropaenic Patient: This group of immunocompromised patients require an increased serum bacteriocidal titre for equivalent antibiotic therapeutic responses. In one study (36) a bactericidal titre of > 1:16 resulted in an equivalent clinical response to that achieved with a titre of 1:8 for Gram negative septicaemia in non neutropaenic patients. Animal experiments measuring PAE have shown equivalent duration of effect in neutropaenic patients (27)(29). However there is some evidence of bacterial regrowth with potential for treatment failure if serum levels are below MIC for prolonged periods of the dosage interval (3).

iii) Treatment Recommendations.

Interval therapy with 2 - 4 equal IV infusions of amikacin per day has been standard therapy. Recent work has however suggested that once daily therapy using an equivalent total dose may be equal or even more efficacious with decreased toxicity and expense. Dosage recommendations and therapeutic target levels have also been increased especially for compromised patients with serious

infection.

a) The Once Daily Dose: The theoretical basis for daily use of aminoglycosides is based on the role of the PAE, increased bactericidal activity after higher doses and the effect of tissue drug accumulation. Due to aminoglycoside concentration in renal tissue, there is a large experience of successful treatment of urinary tract infection in humans using single daily doses of aminoglycosides (5)(37). Treatment of Gram negative infection in animals has shown that equal (2) or improved results (10) can be achieved with single or multiple dose administration as compared to continuous infusion of the same total daily dose.

A study using netilmicin to treat Gram negative bacteraemia randomised 70 patients with normal renal function to either single or multiple dose therapy using 6 mg/kg/day (30). Equal efficacy and safety was reported. Single daily dose therapy has also been used effectively for treatment of Gram negative pneumonia in cystic fibrosis (3). This is supporting evidence for the proposed theoretical advantage of enhanced penetration of broncho-pulmonary secretions at the high peak levels achieved.

There is concern that prolonged periods of sub MIC serum drug levels may cause break through bacteraemia especially if peak level: MIC < 1:8 (15)(16)(38). This issue is controversial (23) and acquires particularly importance in considering use of single dose daily therapy in seriously ill patients especially those with neutropaenia infected with Pseudomonas or Serratia species (31)(39). While single daily dose therapy has been shown to be effective in the neutropaenic mouse thigh model (31), extrapolation to human disease is not necessarily valid. No human clinical trials have been reported.

A multiple dose form of aminoglycoside therapy as used in this study thus has a rational basis according to available data.

b) Target Therapeutic Levels: A proven strategy for targeting therapeutic levels remains elusive. Efficacy depends principally on peak levels. Trough levels are of little demonstrated significance and are not reliable indirect indicators of the proportion of the dosing interval that the drug level is below the MIC. The effective serum bactericidal titre for aminoglycosides is significantly higher in neutropaenic patients and thus higher peak levels are required for equivalent therapeutic effect.

The target therapeutic peak level in this study was 30 +/- 10 mg/l which is believed on the basis of the literature reviewed, to represent effective therapy in the study population. There is however no definite empiric basis for believing that this represents the optimal therapeutic target. Rapid achievement of target levels was also a therapeutic goal.

1.4.2. AMINOGLYCOSIDE TOXICITY

The principal limitation of aminoglycoside therapy is toxicity. Ototoxicity and nephrotoxicity cause significant morbidity and nephrotoxicity can contribute to mortality in seriously ill patients.

The kinetic - toxic relationship for aminoglycosides is poorly defined and remains controversial. This is partly explained by both interpatient variability in kinetics and susceptibility to toxic effects as well as the inability to accurately predict drug accumulation in target organ tissue. Ototoxicity research is also hindered by the absence of a good animal model of cochlear and vestibular damage. Clinical studies are limited by the difficulty of obtaining cooperation from seriously ill patients and the problem of background noise in the typical ICU environment.

Data on the correlation between serum drug levels, duration of

therapy, concomitant pathological and physiological factors and drug toxicity is reviewed in this section.

i) Ototoxicity

a) Mechanism: High concentrations of aminoglycosides in perilymph and endolymph cause potentially irreversible damage to cochlear and vestibular hair cells. The drug diffuses readily into peri and endolymph but elimination is relatively slow, allowing drug accumulation to a degree proportional to the area under the serum concentration - time curve (AUC) (3)(6).

The exact mechanism of hair cell damage is uncertain. Aminoglycosides inhibit the hair cell membrane Na+ - K+ ATPase which alters membrane potential (6). Binding to cell membrane phosphoinositol specific phospholipase C causes inhibition of prostaglandin production and the resultant decreased levels of cytoprotective prostaglandins may facilitate endotoxin mediated cytotoxicity (6)(40).

b) Clinical Features: The outer hair cells mediating high frequency (> 4000 Hz) sound reception are affected first (3). This is usually asymptomatic but may result in tinnitus or a feeling of fullness in the ears. The deficit is readily detected by tone audiometry and usually reverses on cessation of aminoglycoside use (41).

Subsequent destruction of hair cells mediating low frequency hearing results in a clinically detectable deficit which is usually bilateral, symmetrical and irreversible (3). Hearing deficits usually become apparent during therapy but have been documented to occur up to 6 weeks after the cessation of drug use (3).

Vestibular damage tends to parallel cochlear damage. Clinical features include vertigo, dizziness, nausea and nystagmus. Damage

is usually reversible and compensation occurs through the use of visual mechanisms. Profound deficits occur with increased frequency in the elderly (3).

c) Incidence: A wide range of incidence of toxicity is reported. This reflects variability in both the definition and measurement of ototoxicity as well as differing susceptibility between groups studied.

The reported incidence of clinically evident ototoxicity incidence ranges from < 1% (15) to 0.5 - 5% (6) and 2 - 10% (3).

Audiometrically diagnosed ototoxicity defined either as an increase in hearing threshold of > 15 or > 20 dB occurs with an incidence ranging from 22% (40) to 43% (3).

d) Risk Factors: 1) Drug related: Ototoxicity correlates well with the accumulation of drug in the deep tissue compartment. This is well represented by the area under the serum concentration -time curve (10), the cumulative total dose (16)(28)(40) and the duration of therapy (10)(13)(16)(28)(40). Increased amikacin ototoxicity occurs when the cumulative dose exceeds 15 g or treatment continues for > 10 days (41).

Total dose and treatment duration guidelines are of limited value in treating seriously ill patients requiring aminoglycoside therapy. Manipulation of individual doses to achieve non toxic drug levels is however possible.

A significant correlation between elevated trough levels and ototoxicity exists (3)(16)(40)(41)(42) and one study (13) using multivariate analysis identified an elevated trough level as the single most important determinant of ototoxicity.

The evidence for a significant correlation of peak level with ototoxicity is less convincing. An increased risk of ototoxicity correlating with a peak amikacin level > 32 mg/l has been reported (3)(41)(42). This finding is not substantiated by other studies

with no increase in toxicity being reported for peak levels maintained in the range 20 - 40 mg/l (40) and multivariate analysis failing to establish peak levels as a independent risk factor for ototoxicity (13). Massive single aminoglycoside overdose does not cause ototoxicity (10).

There is limited evidence that continuous infusion is more ototoxic (43).

The ototoxicity risk for amikacin tobramycin and gentamicin is believed to be equivalent with unsubstantiated evidence for decreased toxicity risk for netilmicin (3).

2) Non Drug Related: Age has been shown in one study to be a significant risk factor on multivariate analysis (16). This is not however supported by the findings of other studies (41)(42).

The presence of bacteraemia and high peak temperature correlates significantly with ototoxicity on multivariate analysis (16)(28)(40).

The role of renal function impairment as a risk factor for ototoxicity is controversial. Although there are reports of significant correlation between the degree of functional impairment and the risk of ototoxicity (28), no correlation was found when therapeutic drug levels were maintained (40)(41).

Hepatic failure is a significant risk factor for ototoxicity (40).

e) The Value of Therapeutic Drug Monitoring: The evidence suggests that use of TDM to avoid toxic trough levels may decrease aminoglycoside ototoxicity.

ii) Nephrotoxicity

a) Mechanism: The role of aminoglycosides in the aetiology of renal failure is difficult to discern from numerous other insults to renal integrity in the seriously ill patient. Aminoglycosides are toxic

to the epithelial cells of the proximal renal tubule. The drug binds to the brush border phospholipid and is then taken up into the cell by pinocytosis (6)(31). Intracellular fusion of the pinocytic vesicles with primary lysosomes then occurs. Lysosomal phospholipase and sphingomyelinase is inhibited resulting in lysosomal phospholiposis and accumulation of myeloid bodies as well as release of toxic hydrolases into the cytoplasm (6)(31). Injury is manifest by loss of the brush border microvilli followed by swelling and disruption of cellular organelles and then cell necrosis and sloughing into the renal tubules to form cellular casts (6). The basement membrane is preserved and regenerative activity occurs when the insult is removed with full functional recovery being usual (3)(6).

b) Clinical features : Renal impairment typically presents as acute non oliguric renal failure within 5 - 7 days of the start of drug exposure. There is a marked decrease in concentration ability and glomerular filtration rate and increased serum creatinine levels (3)(6).

c) Incidence : A wide range of incidence of nephrotoxicity for aminoglycosides is reported. This is due to the absence of standard definitions for renal failure, the coexistence of multiple nephrotoxic factors in many patients, variable patient susceptibility to nephrotoxic factors and a poor correlation between renal creatinine and aminoglycoside handling. The serial assessment of renal function and nephrotoxicity is complicated by changes in renal blood flow secondary to variation in cardiac output during fever and infection. The resolution of infection decreases cardiac output and renal blood flow and the associated decrease in glomerular filtration may then be ascribed to drug toxicity. Furthermore most studies require patients to be on aminoglycosides for a minimum of seven days before changes in renal function are

considered relevant. This causes considerable bias towards selection of more seriously ill patients who are more likely to have deteriorating renal function due to factors relating to the underlying disease process. The histological features of aminoglycoside toxicity are those of acute tubular necrosis which are indistinguishable from those of sepsis and shock.

Nephrotoxicity is usually defined for study purposes as an increase of serum creatinine of 43 umol/l (0.5 mg%) above the base line and/or a decrease in calculated creatinine clearance of 50% or more that is associated with aminoglycoside use. Measurement of urinary casts, urine renal enzyme levels and B microglobulin levels to define renal damage is sensitive but lacks specificity (6)(28).

The range of incidence of nephrotoxicity has been reported at 0.5 - 63% (6). Large prospective studies have reported incidences of 7.2% (44) and < 1% in 1640 patients with individualised doses and controlled levels (15).

d) Risk factors : 1) Drug related: Nephrotoxicity correlates to drug concentration in the renal cortex (31) which in turn depends on the extent of drug exposure and rate of uptake.

Renal toxicity risk has been associated with total dose (28), duration of treatment (16)(28)(44), exposure to aminoglycosides in the previous 70 days (3)(16), peak and trough levels (16) and peak level only (16). However, only the duration of treatment is a proven risk factor on multivariate analysis (44). There are conflicting reports that duration of therapy (37) and peak and trough levels (16)(44) are not independent risk factors for nephrotoxicity. It is suggested that high trough levels may be more important as indicators rather than predictors of nephrotoxicity (10)(39)(45).

A 1976 study (43) reported a higher frequency of nephrotoxicity with intermittent as compare to continuous therapy. However, animal

studies have subsequently shown that continuous infusion results in significantly higher renal cortical levels for both amikacin and gentamicin than single daily injection of the same dose (31). Lower renal cortical levels for intermittent dose therapy was also reported from a clinical trial on patients prior to nephrectomy (31). It is thus likely that less frequent administration of larger doses results in less renal cortical drug accumulation. The renal toxicity of currently used aminoglycosides i.e. amikacin, gentamicin, tobramycin and netilmicin is generally believed to be equivalent (3)(28)(37). However there is an experimental basis for claims of variable drug toxicity. Renal tubular cell uptake kinetics have been described in one report as linear for amikacin but saturatable for gentamicin, tobramycin and netilmicin (16) while another study (31) reported linear uptake for tobramycin, intermediate uptake for amikacin and saturatable uptake for gentamicin and netilmicin. It is possible that the uptake pattern may have a significant influence on nephrotoxicity if larger doses are administered less frequently to achieve the same daily dose of drug.

2) Non drug related: Increased age predisposes to aminoglycoside nephrotoxicity (3)(16)(37)(39)(44).

Animal experimentation suggests decreased risk in females (37). Some clinical studies report an increased risk in females (16)(28)(39) but multivariate analysis (44) suggests that males may be at increased risk.

Shock and dehydration are risk factors for nephrotoxicity (3)(28)(37)(39)(44).

Infection has been reported as a significant risk factor (3). Accurate assessment is difficult because resolution of fever results in decreased renal blood flow and glomerular filtration rate and this phenomenon cannot easily be distinguished from drug related

effects.

Earlier studies (3)(44) suggested an increased risk of nephrotoxicity in patients with compromised renal function. More sophisticated studies using multivariate analysis and maintaining drug levels within a therapeutic range support the concept that higher pre administration GFR results in increased exposure of target tubular cells to the drug in glomeruler filtrate. This is believed to enhance nephrotoxicity (16)(38)(46).

Hepatic dysfunction may cause renal vasoconstriction with decreased renal blood flow and elevated plasma renin levels. Liver disease has been proposed as a significant risk factor for nephrotoxicity in several studies (16)(37)(39)(44) (46).

Data on additive nephrotoxicity caused by concomitant use of other drugs remains conflicting. Loop diuretics, first generation cephalosporins and vancomycin may enhance aminoglycoside nephrotoxicity (28)(39)(44). There is a widely held belief that the nephrotoxicity of cyclosporin, acyclovir and amphotericin B may be additive to that of aminoglycosides. However, some studies (37) have failed to prove a significant correlation between the use of furosemide and cephalothin and risk of nephrotoxicity.

The drug and non drug related correlates of aminoglycoside nephrotoxicity remain uncertain. This is especially evident in the failure of several attempts to develop a predictive algorithm for nephrotoxicity (6)(46).

e) The value of TDM : A retrospective case controlled study on 313 patients (44) demonstrated a significant decrease in gentamicin nephrotoxicity with strict drug level control.

A randomised prospective double blind study comparing the use of tobramycin and gentamicin in 214 patients (37) demonstrated a decreased risk of nephrotoxicity when drug levels were maintained

within a therapeutic range. This beneficial effect was most significant for patients with impaired renal function.

A study of 103 cases of amikacin therapy in granulocytopaenic patients in whom drug levels were controlled revealed no evidence of increased nephrotoxicity despite the use of significantly higher doses to achieve target drug levels (47).

A review article on aminoglycoside toxicity (6) concluded that TDM results in a significant decrease in gentamicin nephrotoxicity. However, careful monitoring of serum levels does not exclude the risk of nephrotoxicity (16).

iii) Conclusion

The most important determinants of drug toxicity are total dose and duration of therapy. This knowledge is of limited therapeutic value because these parameters cannot be prospectively controlled so as to decrease toxicity. Drug levels are however readily available and drug dose and dose frequency can be altered. The correlation of trough levels with toxicity is important because control of levels can present a therapeutic goal. The value of peak level control for decreasing aminoglycoside toxicity is less well established. Single daily dose would also appear to minimise toxicity.

In this study control of trough levels was implemented with an upper limit of 6 mg/l for amikacin being used. The upper limit for peak levels was set at 40 mg/l. Due to concern about therapeutic efficacy, single daily dose therapy was not used except in patients with impaired renal function.

1.5. THE THERAPEUTIC ROLE OF DRUG MONITORING

1.5.1. MEASURING AMINOGLYCOSIDE LEVELS.

The use of rapid and accurate aminoglycoside assays has made drug monitoring possible. Aminoglycoside levels were originally assayed using a slow and cumbersome bioassay. This was replaced by accurate but labour intensive high performance liquid chromatography and gas liquid chromatography methods. Clinical assays currently use either the radioimmunoassay or fluorescent antibody immunoassay. The fluorescent antibody method is more popular because it yields good interassay correlation and minimal bias and avoids radiation exposure (3).

The fluorescent polarization immunoassay (TDX ABBOTT) (48) is a widely used assay system which utilizes a fluorophore comprising the specific aminoglycoside bound to a fluorescent dye. This complex is excited by a blue (485 nm) light from the polarized light source, and emits a green (525-550 nm) light on return to the steady state. The polarization of emitted light depends on the position of the fluorophore at the time of photon emission. If the fluorophore is bound by a large antibody molecule it can no longer rotate freely and the emitted green light is in the same plane as the blue excitation light i.e. polarization is retained. If the fluorophore is unbound it is free to rotate and can emit the green light in a any plane i.e. polarization is lost. The TDX competitive binding method allows tracer labelled aminoglycoside and patient serum aminoglycoside to compete for binding sites on a fixed number of the anti-aminoglycoside antibody molecules. Since emitted light polarization increases as molecular size increases and molecular size is dependent on antibody binding, a precise relationship between the drug level in a specimen and polarization can be established by measuring the polarization of calibration specimens

of known concentration and establishing a standard curve. Polarization measurements from specimens of unknown drug concentration can then be correlated with drug levels.

1.5.2. EMPIRIC DRUG USE.

The availability of rapid and reliable drug assays allows therapeutic monitoring of drug levels. It was soon demonstrated that use of the mg/kg dose recommended by the drug manufacturers resulted in unpredictable and often non therapeutic drug levels (3). Empiric (trial and error) therapy in 86 patients without organ failure and who did not require ICU admission (8) resulted in 21% toxic and 36% sub therapeutic levels. A randomised prospective study of 40 acutely ill patients (49) compared clinician estimates of dosage requirements with the calculated dose using a nomogram and the predictions of a kinetic program. Use of the empiric method resulted in 20% subtherapeutic peak and 70% toxic trough levels as compared to 85% of trough levels and 90% of peak levels in the therapeutic range for the kinetic method. Nomogram based therapy was not significantly more accurate than the empiric method.
A survey of aminoglycoside levels achieved in the Isolation Unit of the Department of Haematology at Groote Schuur Hospital was performed in January 1990 (Groenewald P. et al personal communication). No attempt was made to supervise or monitor drug administration or specimen sampling which was performed routinely by nursing staff. Results can thus not be used for comparison with other methods of TDM but are valuable in demonstrating the performance of the empiric method in this Unit. Subtherapeutic peak levels (< 20 mg/l) occurred for 49% of levels and 16% of trough levels were toxic (>6 mg/l). Only 46% of paired peak and trough levels were therapeutic (peak 30 +/- 10 mg/l and trough not >6 mg/l).

Empiric aminoglycoside therapy by experienced medical staff does not consistently achieve therapeutic and non toxic drug levels.

1.5.2. NOMOGRAMS.

Patient physiological parameters including age, sex and calculated creatinine clearance may be used in an algorithm to calculate individual aminoglycoside requirements. This method depends on several assumptions which are not necessarily valid for aminoglycoside kinetics especially in infected patients. Vd is presumed to be a constant and predictable fraction of actual and/or ideal body weight. Drug clearance is calculated from the serum creatinine or creatinine clearance value.

Although nomogram predictions have been shown to achieve up to 85% accuracy for therapeutic levels in selected male patients under 50 years old with no organ failure and treated outside of ICU (46), general use in non selected populations has proved disappointing with achievement of only 48% therapeutic range peak with 30% toxic trough levels reported in one review (3). A further study reported the achievement of therapeutic peak and trough levels in only 30% of cases (50).

Nomograms retain a role for initial dose calculation in the Bayesian kinetic prediction programs.

1.5.4. THE KINETIC PREDICTION MODEL.

Measured drug levels are used to construct a concentration - time curve. Kinetic parameters for the individual are derived and dose requirements to achieve target of drug levels can then be calculated. Several individualized kinetic prediction models have been developed. This discussion is limited to two, that of Sawchuck and Zaske (SZ) and the Bayesian statistical method. Calculation of a required dose from derived kinetic parameters

utilizes standard kinetic formulae.

i) The Sawchuck Zaske Method.

This is based on a one compartment kinetic model with drug level measurement being delayed to at least one hour after the start of infusion to avoid inaccuracies due to the initial distribution phase (3). The peak level achieved is largely a function of Vd and this level is thus an important data point for Vd estimation. Measurement of a second level at approximately 1.5X the usual drug half life yields an accurate estimation of graph slope and thus drug clearance. Data points in excess of 4 per interval yield little additional information. No patient physiological parameters are required. The serum concentration -time data is fitted to a regression curve using a least squares analysis. The most accurate results are achieved using computerized non linear regression analysis.

The S-Z method has been well validated for several groups of patients in numerous clinical trials and has made a considerable contribution to effective therapeutic drug monitoring.

Cipolle et al (18) studied the clinical efficacy of individualised tobramycin therapy in 26 patients with serious Gram negative pneumonia. Dosage requirements showed significant inter- and intrapatient variations with a range of 0.4 - 15.5 mg/kg/day. Optimal serum levels were achieved early in the treatment course and were well maintained. There was no nephrotoxicity nor clinical ototoxicity. The authors concluded that the high clinical response rate of 88% was significantly influenced by the maintenance of therapeutic drug levels.

Zaske et all (51) studied the use of the S-Z method for gentamicin therapy in 66 patients with severe burns complicated by Gram negative sepsis. Individualised therapy was shown to be a significant factor in the survival for the first episode of sepsis

with an improvement from 33 to 64% using a historical control. Larger doses of drug were required in the individualized therapy group i.e. 7.4 mg/kg/d vs 3 - 5 mg/kg/d. Wide inter- and intra-patient variation in dosage requirement was shown.

Sveska et al (52) reported a retrospective review of differences in outcome between individualized and non individualized aminoglycoside treatment of culture proven Gram negative pneumonia or sepsis in 42 patients. The authors concluded that individualized therapy is associated with decreased mortality, a decrease in the rise of serum creatinine level above base line and decreased length of drug therapy and hospital stay.

Denaro and Ravencroft (11) reported a prospective study to determine the prediction performance and therapeutic value of individualized drug therapy in 51 patients. The method predicted 79% of peak concentration values within 2 mg/l of the target level of 8 mg/l for tobramycin and gentamicin and 82% of trough levels within 1 mg/l of target trough levels of 1mg/l. Significantly increased doses i.e. 1.5 - 2 X recommended, were required to achieve therapeutic levels. A wide variation in kinetic parameters was reported, Vd 0.16 - 0.52 l/kg and clearance 0.04 - 0.17 l/kg/hr despite normal initial serum creatinine levels in all patients. The authors concluded that individual kinetic parameter determination is essential for early achievement and maintenance of therapeutic drug levels.

Reed et al (12) reported a prospective study on the use of nephrotoxic antibiotics in 166 patients in a surgical ICU. Significantly improved therapeutic peak level frequency was achieved for individualised therapy compared to a control group. Therapeutic gentamicin peak levels were achieved in 9% of controls as compared to 91% of individualized patients ($P<0.005$) with the corresponding figures for tobramycin being 27% vs 92% ($P<0.0001$). Critically ill patients required larger doses and longer dosage intervals than

generally recommended. Large intrapatient kinetic variability was observed and the authors suggest a minimum of alternate day measurement and kinetic prediction with dose adjustment as necessary. The authors rejected the often stated belief that 5X drug t 1/2 is required to achieve "a steady state" and measured levels after both the initial dose and immediately following dose adjustment.

El-Sayed and Islam (49) compared non kinetic and kinetic approaches to individualisation of gentamicin dosage in 40 acutely ill patients. Use of the non kinetic method resulted in 70% toxic trough levels and 20% sub therapeutic peak levels, while the kinetic method (SZ) achieved an 85% therapeutic trough and 90% therapeutic peak level.

ii) The Bayesian Method.

Bayes theorem converts prior probabilities into posterior probabilities by their interaction with likelihoods. Prior probability can be ascertained or estimated from retrospective observations in the specific population from which a subject is derived. In drug kinetic studies the prior probability is the known mean and standard deviation of kinetic parameters such as Vd and Cl. A new measurement or observation can then be used to estimate a revised value for the parameter which is then known as the posterior probability (53).

Bayes theorem utilizes population and individual parameter measurements to obtain reliable estimates of individual kinetics (54)(55). Model initiation assumes that an individual is a typical member of a population with known kinetic parameters. This assumption is optimal prior to drug use because no information is available to estimate the patients individual variation from the mean parameters corrected for that individuals physiological variables such as age, weight, sex and creatinine clearance.

Measured drug levels at known times after administration of a specified dose are used in an adapted Bayesian formula to adjust the prior probability distribution of an individuals parameter set and thus arrive at a revised or posterior distribution. This posterior distribution is then utilized as a prior distribution for the next round of kinetic forecasting. Thus kinetic parameters are constantly revised to more closely approximate the individual's true kinetic values and to compensate for variations in parameters with time.

The Bayesian method thus adjusts estimates of kinetics after each measurement input so as to maximally utilize available population and patient specific information allowing the estimate of individual parameters to move smoothly from those appropriate to a typical member of the sub population to those appropriate to an individual as information on that individuals characteristics accumulates. The method has been computerized and several versions are available for use on a personal computer. The OPT program was used for this study (56). Kinetic predictions are based on a one compartment open model. The parameters utilized are starting concentration at initialisation of the model, drug clearance and volume of distribution. First order kinetics are assumed and OPT is considered equally valid for steady and non steady state data. The program has been validated for accuracy in several hospital populations (56).

Use of the Bayesian method based individualized drug therapy is well documented and comparison with the S-Z method has been reported. The following is a review of some available information. Burton et al (57) assessed the accuracy of a Bayesian method in providing dosage regimens to achieve desired aminoglycoside concentrations in a prospective study on 78 patients. The method performed well in clinical practise and was significantly more

accurate than either the empirical or nomogram based therapy. There was no significant difference between predicted and achieved peak and trough levels using the Bayesian method for gentamicin and tobramycin. This was compared with the nomogram and empiric methods by determining the ability to predict levels within +/- 2 mg/l for peak and +/-1 mg/l for trough values. The Bayesian method achieved the target for 86.1% of peak and 86.2% of trough levels compared to 63.9% and 75.9% for the nomogram and 33.3% and 66.7% for empiric methods respectively.

In a prospective study in 13 patients (20) Chrystyn assessed the value of Bayesian analysis in optimizing gentamicin therapy from the time of commencement of treatment. The sampling time was at 1 hour and 4 hours after the start of the drug infusion. Results were equal in accuracy to those achieved with the S-Z method using three levels. This study concludes that a one compartment model using two drug levels gave results sufficiently accurate enough for clinical use.

Lacarelle et al (58) evaluated a Bayesian method of amikacin dosing in ICU patients with normal or impaired renal function. The population kinetic parameters were derived from kinetic studies in a population undergoing treatment in the same unit. Bayesian estimates used peak and trough levels. The study concludes that the Bayesian method performance is accurate for both estimates of kinetic parameters and prediction of drug levels with both results and their 95% confidence levels reported below:

	Peak	Trough
Bias (ME) (mg/l)	- 0.03 (-0.68 to 0.60)	- 0.21 (-0.51 to - 0.08)
Precision (RMSE) (mg/l)	2.69 (2.07 to 3.19)	1.24 (0.9 to 1.5)

Prediction precision was better for peak than trough levels. This

reflects the close relationship of peak levels to Vd which tends to fluctuate less than the Cl which correlates more closely to trough levels. The one compartment model was found to function adequately and data routinely collected by unsupervised ICU nursing staff was reliable. The mean Vd was higher than that generally reported at 0.35 l/kg.

Rodvold et al (59) reported on a retrospective evaluation of the predictive performance of a one compartment Bayesian forecasting program in 30 ICU patients with stable renal function. This study demonstrates that the use of population kinetic data derived from ICU patients rather than the general patient population, results in significantly improved prediction precision with less bias. Replacement of general population data with ICU derived population data improved the achievement of therapeutic range levels from 80% to 100%. The use of conventional population data generally resulted in over prediction of peak and trough levels. There was no advantage in using "steady state" levels. Use of further levels in addition to peak and trough levels did not significantly increase the predictive value.

Godley et al (60) compared the predictive performance of three microcomputer programs for gentamicin in a retrospective study on 30 patients. Equivalent performance was found for the three programs using peak and trough levels to predict levels five days later. Performance was matched by the use of the S-Z method. Bayesian methods were found to tend to over estimate drug levels while the S-Z method was biased towards under prediction. One of the Bayesian methods was found to have equivalent prediction accuracy using only one level input.

Bottger et al (42) compared the performance of the Bayesian and S-Z methods in 19 critically ill patients. Both methods resulted in precise and unbiased predictions with the Bayesian method being more

precise for amikacin. The variation in dose requirement for amikacin was 3.8 - 30.0 mg/kg/d. The authors noted that the major factor limiting the predictive accuracy of currently available methods is the large and rapid intrapatient changes in kinetic parameters.

A further comparison between the Bayesian and S-Z methods is reported by Denaro and Ravenscroft (61). 36 seriously ill patients were studied to compared the 4 level input S-Z method with the Bayesian method using trough and from 1 - 3 post infusion levels. All methods performed well except for use of the Bayesian model with only one level measurement which was not successful. There was no significant difference between the S-Z method and the Bayesian predictions which used trough and 2.2 hr post infusion levels. The 4 level Bayesian method yielded the most precise predictions. Bayesian methods tended to under estimate Vd and Cl resulting in level predictions which exceeded those achieved.

iii) Selecting a TDM Method.

The available data suggests that it is possible to achieve accurate kinetic predictions in clinical practise. This should allow dosage prediction and the consistent achievement of therapeutic drug levels. It is also evident that the currently available methods have performance limitations imposed extrinsically by variability in kinetic parameters and the potential for improvement of accuracy is thus limited. Selection of a TDM method to be used depends on accuracy, efficiency and ease of application.

The SZ and Bayesian methods can achieve equivalent predictive accuracy. While the Bayesian method can be used with fewer drug samples and a more flexible sampling regimen, the SZ method has the advantage of requiring no physiological data input. However lack of such input increases reliance on sample level accuracy with an

increased potential for major error should input data be inaccurate. The choice of optimal sampling time is more important for successful use of the Bayesian method. Peak level sampling at 1 hour allows the use of a one compartment model and yields the most valuable input for estimation of Vd. Optimal sampling time for determining clearance is at 1 half life. This is not known in advance and a level at 1 estimated half life after the peak level is thus used. Assessment of cost effectiveness is of increasing importance. Use of the S-Z method usually incurs the cost of additional samples. The data processing equipment and personnel time use for both methods is equivalent. The overall cost effectiveness of TDM has been studied but no reliable cost effectiveness data from prospective controlled studies with appropriate dose and level control is available (62). However, it is well established that TDM is effective in maintaining drug levels therapeutic and non toxic. Furthermore, performing aminoglycoside assays without TDM is of limited value. Thus the indirect evidence is strongly in support of the belief that TDM is cost effective in improving the quality of patient care (63).

This study used a Bayesian method and two aminoglycoside levels per kinetic estimation.

1.6. ANTIBIOTIC THERAPY IN THE FEBRILE NEUTROPAENIC PATIENT.

Infection is the principle cause of morbidity and mortality in the neutropaenic patient. A major contribution to improved treatment results has been effective antibiotic therapy. The definition, aetiology and treatment options for febrile illness in patients with malignancy and neutropaenia are reviewed so as to examine the role and requirements for aminoglycoside therapy in this population.

Both malignant disease and the currently used treatment modalities result in bone marrow suppression and neutropaenia. This results in profound immune compromise and the increased risk of overwhelming infection. The incidence of infection increases significantly when the combined neutrophil and band form count drops below 1 X 10^9/l. Risk of infection increases with decreasing neutrophil counts and life threatening bacteraemia occurs most frequently when counts drop below 0.1 X 10^9/l (64), when the rate of decrease is rapid and if neutropaenia persists for longer than 14 days (36)(65)(66).
Fever is the only reliable indicator of infection in the neutropaenic patient. The definition of fever is arbitrary, but The National Cancer Institute diagnostic criteria (66) are representative of current practise. Fever is considered significant if either 1) 3 oral temperatures exceed 38 degrees C during a 24 hour period or 2) a single temperature over 38.5 degrees C occurs. Infection in the neutropaenic patient has a characteristic distribution with more frequent involvement of the peridontal and pharangeal areas, facial sinuses, oesophagus, colon, rectum and skin and puncture sites. Pneumonia and septicaemia are the most common causes of death (36). Neutropaenia results in decreased inflammatory response to infection which delays both early clinical diagnosis and localization of infection sites. The infecting organisms multiply and spread more rapidly (36) resulting in overwhelming sepsis and rapid demise. Neutropaenic patients have a significantly increased risk of infection with Gram negative aerobic bacilli and Staphlococci complicated by the later development of fungal or bacterial superinfection. The aetiology of infection in a particular group of neutropaenic patients depends on their endogenous flora, 50% of which is usually hospital acquired at the time of infection (66). Organism distribution is largely

influence of antibiotics is demonstrated historically by the ongoing changes in the pattern of infective organisms in febrile neutropaenic patients throughout the period that antibiotic therapy has been available. In the pre-antibiotic era the most common pathogens were Streptococcus pyogenes and pneumococcus. Penicillin use significantly increased Staph. aureus isolates and the subsequent development of anti staphylococal penicillins and cephalosporins in the late 1960s was associated with a significant increase in the prevalence of Gram negative aerobic infection. Use of aminoglycosides and increasingly broad spectrum B-lactams in the 1970s resulted in the emergence of an increasing problem with Gram positive cocci including Staph. aureus and epidermidis, group D Streptococci, B haemolytic Streptococci as well as diptheroids and anaerobes. The spectrum of Gram negative infection also shifted to include more isolates of citrobacter, acinetobacter and enterobacter (67). The increased incidence of Gram positive cocci and especially staphylococci may partly be ascribed to monotherapy with cephalosporins (68), prophylactic use of quinolones (66) and increased use of indwelling vascular access devices (68).

Febrile episodes are clinically ascribed to and treated as infection in neutropaenic patients although 25-40% (36)(66) of cases have no microbiological or clinical basis for this diagnosis. Many of these febrile episodes may be due to transfusion reactions, drug fever, cell lysis following chemotherapy and fever associated with the underlying malignancy. The overall rate of isolation of an infective cause of fever from blood or other sites ranges from 6-40% (64)(69). This low rate of organism isolation implies that reported isolates do not necessarily represent the cause of infection in this population.

No data is currently available on the spectrum of isolates from the Unit in which this study was performed. Use of published data is

thus required for the planning of therapy. The European Organization for Research on Treatment of Cancer (EORTC) has published three large multicentre survey based reports on the microbiological isolates from neutropaenic patients with fever. The EORTC I trial of 1978 reported a 20% incidence of documented bacteraemia in febrile patients with malignancy and neutropaenia. These were equally divided between Gram positive and negative isolates (64). The EORTC III trial of 1982 revealed an increased proportion of Gram negative isolates at 57%. Later reports especially those from North America reveal a greater frequency of Gram positive organisms averaging 60% (70). However mortality rates for Gram positive infection remains lower at 5 -23% (36)(70) compared to a 30 - 40% mortality for Gram negative infections (70).

Empiric therapy should thus include effective cover for Gram negative infection which remains a serious threat to life.

1.6.1. THERAPEUTIC GOALS.

Neutropaenic patients with fever require prompt therapy. Empiric antibiotic therapy is accepted universally as a major factor in improving survival although this has not been verified in a controlled study (71). The historical precedent of the very poor results when commencing antibiotic therapy only after documentation of infection (64) is accepted as adequate proof of efficacy for current practice.

Although antibiotics have an important role, the most significant survival and prognostic factor in this group of patients is recovery of adequate leucocyte function (72)(73). Severe neutropaenia is a major risk factor for treatment failure caused by superinfection (36).

Optimal use of antibiotics depends on rapid delivery of adequate amounts of the correct drug to the site of infection while avoiding

undue toxicity. Knowledge of the type of infecting organism and its in vitro sensitivity is rarely available at the initiation of therapy. Empiric antibiotic therapy should thus cover the most probable infected organisms while minimizing disturbance of colonizing flora and the development of resistance. Combination therapy using an extended spectrum B-lactam and aminoglycoside is widely used but not universally accepted therapy.

1.6.2. SYNERGISTIC ANTIBIOTIC THERAPY.

Use of synergistic combinations has several advantages:

1) Provision of more efficacious and extended spectrum cover.
2) Prevention of development of resistant organisms.
3) Allows use of lower drug doses with reduced toxic potential.

i) Drug Effectiveness.

The value of empiric combination therapy with extended spectrum B-lactam and aminoglycoside combinations is well established with no particular combination being shown to have a significant advantage (67). Gram negative infection mortality rates of 60 - 90% in the 1950s has been reduced to the current levels of 10 - 30% (67). The enhanced effect of combination therapy is especially well demonstrated for persistently neutropaenic patients in whom survival has increased from 0% to 44% (71). B-lactams in combination with aminoglycosides have been shown to increase the serum bacteriocidal activity from 1:4 to 1:16 with a concomitant improval in favourable outcome from 49 -80% (36). Combination therapy may be effective against organisms resistant to either the B-lactams or aminoglycoside used alone. In one study (36), 70% of Staph. epidermidis resistant to both B-lactams and aminoglycosides were susceptible to the two drugs used in combination. In vitro studies

have shown enhanced Staph. epidermidis killing even at low aminoglycoside levels with a decreased incidence of emergence of resistance (36). Group D streptococci (Enterococci) may be resistant to B-lactams by virtue of decreased affinity of a lower molecular weight penicillin binding protein. Aminoglycosides are clinically ineffective because bacteriocidal drug concentrations cannot be achieved in vivo. However, a combination of B-lactam and aminoglycoside results in sufficient disruption of bacterial cell walls to allow high intrabacterial aminoglycoside levels which are rapidly bacteriocidal (2)(74). Synergistic action against Pseudomonas has been demonstrated but the mechanism of the antibiotic effect remains uncertain (74).

Synergism between different B-lactam antibiotics is currently subject to intense research. While a degree of synergism does occur for some combinations, data remains less certain than that for the B-lactam aminoglycoside combination and the potential for drug antagonism exists (73).

ii) Antibiotic Resistance.

There is some experimental and clinical evidence that combination therapy prevents or retards the emergence of antibiotic resistant strains (36)(72). This has become evident in trials of extended spectrum B-lactams as monotherapy for fever in neutropaenic patients during which resistant Gram negative organisms have caused serious nosocomial infections. Resistance may be due to either the production of induceable cephalosporinase or plasmid mediated B-lactamase. Animal studies have shown that the combination of B-lactams with aminoglycosides limits the emergence of resistance. However a disturbing finding has been that some B-lactam resistance plasmids also coded for aminoglycoside resistance (75).

Aminoglycoside only empiric therapy has been attempted, but had a

50% treatment failure rate principally due to the rapid development of resistant organisms. (43)(67).
Imipenem is the latest agent to be used as monotherapy and aquired resistance among Pseudomonas aeruginosa has emerged as a significant problem (67).

iii) Toxicity.

The principal motivation for the development of alternative non aminoglycoside containing regimens has been the problems of aminoglycoside nephrotoxicity and ototoxicity. Concern has increased with the more frequent use of agents such as amphotericin B, vancomycin and cyclosporin A which are also potentially nephrotoxic.
Several theoretical potential adverse effects of the B-lactam aminoglycoside combinations have been researched. No additional nephrotoxicity has occurred with the addition of third generation cephalosporins to aminoglycoside therapy (36)(74). The chemical inactivation of aminoglycoside by B-lactams has also never been demonstrated as a significant in vivo problem (36)(74).
Significant toxicity does occur with B-lactam combination therapy. A combination of moxalactam and pipricillim resulted in a 48% incidence of detectable coagulopathy and 1% nephrotoxicity compared to 9% nephrotoxicity and 21% ototoxicity for a combination of moxalactam and amikacin reported in the same study (73).

1.6.3. DURATION OF THERAPY.

The optimal duration of antibiotic therapy in febrile neutropaenic patients remains uncertain. Current dogma asserts that antibiotic therapy should not be stopped until neutropaenia has resolved. This is largely based on the results of a single prospective controlled study with limited clinical information and in which antibiotic

therapy was stopped after 24 hours of apyrexia (76). There was a relapse of fever in 41% of patients most of whom responded well to resumption of antibiotic therapy. Further exposure to antibiotic was avoided in 59% of patients despite the cessation of therapy after a very short period of apyrexia. There is also no good evidence that ongoing use of empiric antibiotics in patients with persistent neutropaenia and resolved fever is effective in preventing further febrile episodes (68). Prolonged use of broad spectrum antibiotic therapy increases the risk of toxicity, superinfection, the development of resistant organisms, drug fever and drug allergy (76). It may thus be reasonable to propose cessation of therapy at 7 days after the last febrile episode in a persistently neutropaenic patient (76).
The patients in this study were treated according to Unit protocol with antibiotic therapy being stopped when the neutrophil count was above 1 X 10^9 /l and the patient had been apyrexial for at least 48 hours.

1.6.4. USE OF TDM.

Finley et al (47) have reported a comparison of standard vs pharmacokineticaly adjusted amikacin dosing in granulocytopaenic patients. A non Bayesian kinetic model was used The comparison was done sequentially on different patient groups and was thus neither randomised nor fully controlled. The authors concluded that TDM was a significant factor in the safe use of increased doses of amikacin which contributed to a improval in response rate from 56 to 80%.

1.6.5. CONCLUSION.

Optimal antibiotic therapy for the febrile neutropaenic patient with haematological disease is not yet established. A major limitation to therapeutic efficacy remains the constant evolution of microbial

antibiotic resistance. On the basis of available data it is proposed that febrile episodes are currently best treated by early initiation of therapy with an empiric combination of a third generation cephalosporin or extended spectrum penicillin with an aminoglycoside. Further changes in therapy depend on results of microbial investigations and clinical response. Optimal aminoglycoside therapy can only be instituted and maintained by effective use of TDM. However experience in the use of TDM in this population is limited and no studies using Bayesian models have been reported.

A further contribution to decrease toxicity is limitation of the duration of therapy to the shortest period possible. There is no evidence to support the continued use of antibiotic therapy in patients who have become afebrile until the resolution of neutropaenia.

2. AIM.

To evaluate the use of the OPT Bayesian pharmacokinetic predictive program for estimation of amikacin dose requirements in febrile neutropaenic patients with haematological disease.

3. METHODS.

3.1 PATIENT SELECTION.

The study was conducted in the Isolation Unit of the Department of Haematology at Groote Schuur Hospital (GSH) and the University of Cape Town. This is a referral centre for the Cape Province and is the only Unit offering bone marrow transplantation (BMT) in this region.

Sequential admissions satisfying basic selection criteria were enrolled as study subjects. All patients were admitted for investigation or treatment of an underlying haematological disorder, had a febrile episode satisfying protocol criteria and become neutropaenic at some time during amikacin therapy. Fever was defined by the Unit treatment protocol as a temperature exceeding 38 degrees C for longer than 6 hours or a single spike of 38.5 degrees C that could not be attributed to drug administration or a transfusion reaction. Neutropaenia was defined as a count (including band forms) of less than 1 X 10^9/l

3.2. INITIATION OF TREATMENT.

The initial amikacin dose was decided empirically by the attendant medical staff. No attempt was made to influence the initial dose used.

3.3. DRUG ADMINISTRATION.

Amikacin sulphate (Bristol) was administered intravenously diluted in 50ml of either normal saline or 5% dextrose water. Solutions were made up just prior to use by the Sterile Unit of the hospital pharmacy during the day and by the Unit nursing staff at night and on weekends. The drug was infused through indwelling central venous catheters or peripheral cannulas over a period of 30 minutes. Administration rates were either controlled by electronic drop counters or closely monitored by nursing staff. After completion of the infusion the administration set was flushed with 50 ml of saline with heparin to ensure complete delivery of the dose.

3.4. BLOOD SAMPLING.

Blood specimens for peak level assay were taken at exactly one hour after the commencement of drug administration. Sampling was done either through the non administration lumen of the indwelling central venous catheter or by a peripheral venipuncture. A second level was taken in the first dose interval . The optimal time for this was considered to be 3 hours after the onset of the infusion, but any level taken in this interval was considered an acceptable alternative. Specimens were collected in glass vacuum tubes (Vacutainer) and refrigerated at 4 degree C until assay which was done within 12 hours.. Storage of samples at this temperature up to 48 hours did not affect assay levels when assessed separately for 4 extra specimens taken during this study.

3.5. AMIKACIN ASSAY.

Assay was performed in a routine manner by the technical staff of the Department of Microbiology at GSH using the Abbott TDX polarized florescent antibody method. The instrument was recalibrated prior to

the start of the study and thereafter at one monthly intervals. The medium control (value = 15.0 mg/l) was run with each batch of amikacin assays performed and the results recorded. The assay has a lower limit of sensitivity of 0.8 mg/l, this being considered the smallest value that can be distinguished from 0 with 95% confidence (48). Values of < 0.8 mg/l were entered as 0.4 mg/l for the purpose of kinetic parameter determination.

3.6. PHYSIOLOGICAL DATA.

Patient height and weight were routinely measured on admission by the Unit nursing staff. Serum creatinine is measured routinely on admission and then at least 3 times a week. Assays are done by the Department of Chemical Pathology at GSH using either a Technicon SMAC or Astra Beckman automated analyser both of which utilize the Jaffe picric acid method.

3.7. KINETIC ANALYSIS.

Details of age, sex weight, height, serum creatinine level, amikacin dosing history and the two initial levels attained were entered into a IBM compatible PC loaded with the OPT version 5.1 software package (Clydesoft) used under licence in the Department of Pharmacology. Initial kinetic parameters were calculated for each patient. These were then used in an additional section of the program to predict the consequences of various dosage regimens. Estimates of required dose and dose interval were entered by the operator and the predicted resultant peak and trough levels calculated using the kinetic estimates derived from the Bayesian model. Dose size and frequency were then altered by the operator until predictions satisfying the criteria of a peak level of +/- 30 mg/l with a trough level not exceeding 6 mg/l were achieved. The dose regimen was thus not directly derived by OPT but by an operator using an iterative

process. This system allows for increased flexibility but does mean that prediction accuracy cannot be measured against a standard level.

Dose changes were limited to multiples of 50 mg and the dosage intervals to 6, 8, 12, 18 or 24 + 6x hours.

3.8. THERAPY MONITORING.

The selected dosage regimen was implemented within 24 hours of the initiation of therapy. Amikacin levels were then assayed twice weekly according to Unit protocol and more frequently if there were clinical features or biochemical evidence (serum creatinine increase of 30 mg/l above baseline) of renal function impairment. Trough levels were measured 5 minutes before the next dose and peak levels 1 hour after the start of administration of the dose (given over 30 minutes). Each set of measurements was added to the computer data file and the OPT program used to re estimate the individuals kinetic parameters. OPT uses all available data with a time weighting method giving an increased value to more recent data.

The separate program contained in the software package was then used to predict the required dosage regimen. The new dosage regimen was implemented for the drug dose following that in which levels had been taken.

3.9. PREDICTION ACCURACY.

Accuracy was measured by comparing predicted with achieved results. Bias was measured by calculating mean error (ME) and precision by calculating mean absolute error (MAE) and root squared mean error (RMSE).

The method would be considered valid if the 95% confidence interval (Thompson's test) for mean error included 0 and the MAE and RMSE were < 8 mg/l for peak levels and <4 mg/l for trough levels. These

criteria are consistent with those used in previous studies (11) (57)(58)(59) which use < 2 mg/l for peak and < 1 mg/l for trough respectively for gentamicin and tobramycin. The predictions were further assessed by calculating the percentage of drug levels within the therapeutic range when the predictive method was appropriately used.

4. RESULTS

4.1. PATIENT CHARACTERISTICS.

The OPT kinetic prediction method was implemented for the entire course of amikacin use in 10 sequential admissions to the Unit requiring such therapy according to treatment protocols from 10 June 1990 to 27 August 1990.

Patients population studied represents a wide range of physiological and pathological parameters as shown in table 1.

TABLE 1.

Patient	Age (yrs)	Sex	Weight (kg)	Disease	Serum creatinine Initial (umol/l)	Serum creatinine Maximum	Neutrophil Int. X 10^9/l	Neutrophil Min. X 10^9/l
1)	16	M	54	APL	61	171	0.13	0.03
2)	15	M	54	AA	50	77	0.02	0.02
3)	46	F	56	AML	97	97	0.00	0.00
4)	47	F	53	Myeloma	47	69	0.05	0.05
5)	43	M	62	AML	85	102	0.02	0.02
6)	34	F	57	MDS	71	80	0.63	0.08
7)	19	M	58	ALL	73	73	0.40	0.16
8)	60	M	58	CML	83	136	5.60	0.00
9)	15	F	53	AML	58	66	0.08	0.08
10)	26	M	68	HL	66	151	0.03	0.01

ALL = acute lymphoblastic leukemia
AML = acute myeloblastic leukemia
APL = acute promyelocytic leukemia
CML = chronic myeloid leukemia
HL = Hodgkins lymphoma
MDS = myelodysplasia

Int.= count at initiation of amikacin therapy
Min.= lowest count during period of amikacin therapy

Treatment of the underlying disease in 9 patients was by chemotherapy regimens according to established protocols. Patient 6 received no specific therapy. Three patients (numbers 2, 4 and 10) underwent bone marrow transplantation. There was one patient death during the study, patient 1 failed to respond to chemotherapy, developed disseminated intravascular coagulation and subsequently acute respiratory distress syndrome and died in multi-organ failure.

4.2. AMIKACIN THERAPY.

4.2.1. DRUG SAMPLING.

Initial sampling at 1 hour after the first dose was achieved in all 10 patients. A second level within the first dosage interval was obtained in 9 cases with 1 patient (number 9) having the second level available only after the second dose. The second level of the initial assay pair was taken at an optimal 3 hours after initiation of the drug infusion in 5 cases (Patients 5, 6, 7, 8 and 10).

4.2.2. DRUG ASSAY.

A total of 38 batches of TDX assays was performed. The median control had a mean +/- SD assay value of 14.7 +/- 0.5 mg/l with a range of 13.9 - 15.6 mg/l. The manufacturers recommended range for this value is 15 +/- 1.5 mg/l. (Abbott TDX Kit manual)

4.2.3. KINETIC PARAMETERS.

The individual values are tabulated in appendix 1. These allow estimation of the intrapatient changes in kinetic parameters during therapy. A significant degree of variation during therapy is noted with a maximum percentage change from mean value of Vd of +55% in patient 1 and for Cl of +65% in patient 8.

Mean and SD values for each patient are summarized in table 2. The average mean +/- SD of 305 +/- 48 ml/kg for Vd lies within one standard deviation of 270 +/-60 ml/kg for amikacin reported in

TABLE: 2 PATIENT KINETICS

PT NO	Vd (ml/kg)	SD	Cl (l/hr)	SD	Cl (ml/kg/min)	SD	T 1/2 (hr)	SD
1	432.0	121.0	4.9	1.6	1.5	0.5	4.5	2.7
2	299.0	29.0	8.5	3.6	2.6	1.1	1.6	0.7
3	272.0	30.0	4.6	0.6	1.4	0.2	2.3	0.3
4	285.0	26.0	5.6	1.4	1:7	1.1	2.0	0.5
5	230.0	20.0	4.6	0.7	1.2	0.2	2.2	0.2
6	266.0	67.0	7.9	1.5	2.3	0.4	1.4	0.5
7	327.0	71.0	8.7	2.7	2.5	0.8	1.6	0.5
8	337.0	52.0	3.4	1.4	1.0	0.4	3.6	1.7
9	266.0	20.0	5.5	0.6	1.7	0.2	1.8	0.1
10	339.0	47.0	7.7	2.2	1.9	1.5	1.9	1.1
Average:	305.3	48.3	6.1	1.6	1.8	0.6	2.3	0.8

TABLE: 3 PREDICTION PERFORMANCE

PT NO	DOSE	MEAN PK	MEAN PR	ME	MAE	RMSE	MEAN TR	MEAN PR	ME	MAE	RMSE
1	11.9	34.6	28.1	1.2	6.9	7.9	8.7	3.8	4.9	4.9	6.0
2	11.3	29.3	26.6	2.7	4.5	5.5	2.0	1.2	0.8	1.0	1.4
3	9.3	26.0	27.4	-1.4	4.0	4.6	1.3	1.0	0.3	0.7	0.9
4	11.5	30.3	31.8	-1.5	4.3	5.6	1.2	0.6	0.6	0.7	0.8
5	9.1	28.1	34.8	-6.7	6.7	6.9	2.9	3.8	-0.9	1.4	2.4
6	9.1	30.6	22.4	8.2	8.2	8.5	2.4	1.0	1.4	2.3	2.5
7	11.7	36.6	26.2	10.4	10.4	10.8	2.8	1.4	1.5	1.5	1.9
8	8.6	22.3	25.4	-3.1	4.9	6.0	4.3	2.4	1.9	1.9	2.0
9	9.8	31.8	29.1	2.8	8.4	8.8	1.5	0.4	1.7	1.7	1.7
10	12.2	31.3	29.1	2.3	6.7	9.1	2.2	0.8	1.0	1.5	1.8
Average:	10.5	30.1	28.1	1.5	6.5	7.4	2.9	1.6	1.3	1.8	2.1

TABLE: 4 PREDICTIONS FROM FIRST DOSE

PT NO	PEAK	PR PEAK	ACH-PR	TROUGH	PR TROUGH	ACH-PR
1	46.4	39.7	6.7	12.5	5.1	7.4
2	21.5	25.6	4.1	0.4	0.1	0.3
3	22.9	29.3	6.4	1.1	0.9	0.2
4	24.4	36.2	-11.8	0.4	0.1	0.3
5	29.4	37.9	-8.5	2.6	4.9	-2.3
6	36.5	25.0	11.5	1.8	0.1	1.7
7	34.0	28.7	5.3	2.4	0.1	2.3
8	19.7	24.0	-4.3	3.4	0.5	2.9
9	40.5	29.4	11.1	2.0	0.4	1.6
10	16.3	28.5	-12.2	0.4	0.3	0.1
Average:	29.2	30.4	0.8	2.7	1.3	1.5

standard references (1). The mean +/-SD value for clearance of 1.8 +/-0.6 ml/kg/min also lies within one standard deviation of the value of 1.3 +/-0.6 reported for the general population (1).

4.2.4. OVERALL PREDICTION ACCURACY.

A total of 51 predicted vs achieved peak and trough pairs were studied and the results summarized in table 3.

i) Peak Levels.

Bias is measured by ME = 1.5 mg/l with a 95% confidence interval of -2.6 to 6.5 which includes 0.

Precision is measured by MAE(6.5 mg/l) and RMSE(7.4 mg/l), both have values < 8 mg/l

ii) Trough Levels.

Bias is measured by ME = 1.3 mg/l with 95% confidence interval of 0.4 to 1.8 which does not include 0.

Precision is measured by MAE(1.8 mg/l) and RMSE(2.1 mg/l), both have values < 4 mg /l

4.2.5. FIRST DOSE PREDICTION ACCURACY.

Results are summarized in table 4.

i) Peak Levels.

Bias is measured by ME = -1.3 mg/l with 95% confidence interval of -10.8 to 9.8 which does include 0.

Precision is measured by MAE = 8.2 mg/l and RMSE = 8.7 mg/l, both have values > 8 mg/l.

i) Trough Levels.

Bias is measured by ME = 1.5 mg/l with 95% confidence interval 0.1 to 2.7 which does not include 0.

Precision is measured by MAE(1.9 mg/l) and RMSE(2.9 mg/l), both have values < 4 mg/l.

4.2.6. ACHIEVEMENT OF THERAPEUTIC DRUG LEVELS.

The 51 pairs of amikacin levels reflecting use of dose regimens determined by kinetic predictions were assessed for achievement of

levels within the therapeutic range.
Therapeutic peak levels were defined as 30 +/- 10 mg/l and toxic trough levels > 6 mg/l.

	Therapeutic	toxic	subtherapeutic
Peak:	41 (80%)	4 (7%)	6 (12%)
Trough:	48 (94%)	3 (6%)	Not Applicable

Five patients had all levels based on TDM predictions within the therapeutic range. All 3 toxic trough levels occurred in patient 1. This is partly due to an initial attempt after dose 1 to predict kinetic parameters from a peak level alone. The resultant excessive dose was implemented before kinetic parameters were re estimated using both peak and trough levels. The recalculated parameters predicted the resultant toxic level.
Therapeutic levels were achieved in 7 patients after implementation of the first kinetic prediction based dose. Two patients had toxic and one patient subtherapeutic levels.

5. DISCUSSION

Aminoglycosides are valuable antibiotics in the management of febrile neutropaenic patients with haematological disease. A comprehensive literature review reveals that aminoglycosides retain a principal role with current evidence not supporting their replacement with extended spectrum B-lactams. It is also evident that effective and safe use of aminoglycosides requires early achievement of therapeutic peak levels and the avoidance of high trough levels. Despite increasing evidence for the advantage of once daily aminoglycoside dosing regimens, this is not established for neutropaenic or other compromised patients and on the basis of current in vivo evidence, multiple dose therapy is likely to remain necessary in this population.

Aminoglycoside pharmacokinetics are highly variable and not predictable using conventional clinical and biochemical indicators of patient status. It is thus not surprising that empiric and nomogram based drug use frequently results in non therapeutic levels. In contrast, use of measured drug levels in an individualized kinetic TDM model results in consistent achievement of therapeutic levels even in patients with changing renal function and major extracellular fluid shifts.

The controversy over aminoglycoside kinetic parameter differences in patients with neutropaenia remains unresolved. Seriously ill patients appear to have increased Vd when compared to healthy controls. However it is important to note that the value for Vd has been revised upward in more recent editions of standard references and that values for Vd previously reported as significantly increased in special populations may show no difference when compared to revised values. It is also possible that the increased value for neutropaenic patients may not be significantly different from that for seriously ill patients without neutropaenia. This

study failed to demonstrate a significant difference between the estimates of Vd and CL and those reported in standard references (1). This finding be due either to a failure to detect a difference in kinetics or represent a true finding. There are two major factors limiting the accuracy and value of the kinetic estimates for the population studied. The principal factor is the small population size used. The second relates to study design and the kinetic model used. This study was not designed to determine accurate patient kinetics but rather to assess the clinical value of the OPT program in a specific population. The kinetic results reported are the average obtained for the entire period of treatment for each patient. For several patients this includes a significant period of amikacin treatment while clinically uninfected and apyrexial due to the Unit protocol requirement of resolution of neutropaenia as a condition for cessation of antibiotic therapy. These kinetic parameters are thus not truly representative of the acutely ill neutropaenic patient. The Bayesian model kinetic parameter estimates for a single dose interval cannot be used to compensate for this bias due to the major influence of both population and individual patient prior input. This study does not contribute to resolving the uncertainty about aminoglycoside kinetics in neutropaenic patients.

This study investigated the use of a Bayesian TDM method to implement therapy guidelines. The study was not controlled as the use of TDM is well established. Overall performance of the program in neutropaenic patients is reported using accepted defined criteria. The peak prediction was unbiased and precise with the trough prediction giving biased but precise estimates. Trough estimate bias may be a result of the decision to use the value of 0.4 mg/l for entry into the OPT program when drug assays reported levels of less than 0.8 mg/l. Bias was not of clinical

significance in maintaining trough levels below 6 mg/l.

Initial predictions based on measurements taken after the first dose gave unbiased but imprecise peak level and biased but precise trough level predictions. Trough level bias although statistically significant, did not affect the achievement of non-toxic trough levels in all patients except patient 1. The overall result was the achievement of 80% of peak and 94% of trough levels were in the therapeutic range.

Predictions based on first dose measurements yielded 70% therapeutic peak and 90% non-toxic trough levels.

These results are acceptable and may be used to improved therapy and thus contributing to patient care. However model performance remains suboptimal and a review of the OPT model use may be of value in improving predictive accuracy.

Aminoglycoside kinetic variability imposes an intrinsic limit on the predictive performance of all available programs including OPT. This limitation can only be minimized by a better understanding of the mechanisms of variability. Sampling done by a well motivated and trained nursing staff in an ICU does not appear to be a major source of error The current aminoglycoside assay methods used do not generate significant error. An area of potential improvement is in the initial predictive nomogram section of the OPT program. This is based on population data derived from standard sources at the time of the compilation of OPT (1986), and is unlikely to be representative of the population from which the subjects of this study are derived. Use of that baseline data for a Bayesian program derived from a similar population to that of patients treated results in improved accuracy (58)(59). It was thus decided to reassess the predictive accuracy of OPT using mean values for Vd that are possibly more appropriate for the patient population in this study. Values for Cl and the variance of Vd were not changed.

The values for Vd = 310 ml/kg was derived from the mean value of the kinetic estimate for this study and that of 400 ml/kg as a rounded off average from studies on neutropaenic patient populations reported in the literature (7)(22)(23)(24). Summaries of the results for overall and first prediction accuracy are represented in tables 5 and 6 respectively.

Table 5.

Predictive Accuracy using OPT and altered Vd.

	PEAK LEVEL			TROUGH LEVEL		
	OPT	Vd 0.31	Vd 0.40	OPT	Vd 0.31	Vd 0.40
ME:	1.5	4.2	5.8	1.3	0.9	0.9
MAE:	6.5	6.5	7.3	1.8	1.8	1.7
RMSE:	7.4	7.3	8.2	2.1	2.2	2.1

Table 6.

Accuracy of predictions based on first dose levels for peak values using altered Vd values.

	PEAK LEVEL		
	OPT	Vd 0.31	Vd 0.40
ME:	-1.3	2.31	4.2
MAE:	8.2	6.8	6.8
RMSE:	8.7	8.4	9.1

OPT = value achieved using unaltered OPT program

Vd 0.31 = values achieved for substitution of Vd = 310 ml/kg for initial population mean of Vd.

Vd 0.40 = values achieved for substitution of Vd = 400 ml/kg for initial population mean of Vd.

All values stated are in mg/l.

It is evident from this data that prediction performance was not improved by substitution of revised values for the mean of Vd. However a more substantial revision of program population data including the mean value for Cl and SD of Vd and Cl may be more successful. These manipulations were not performed in this study because of difficulties encountered when attempting to alter the OPT initial population data base file.

TDM resulted in use of a mean amikacin dose of 10.5 mg/kg which is 40% above the manufacturer's recommendation of 7.5 mg/kg/dose for twice daily use. Increased doses were administered without an increased frequency of toxic trough levels in 9 patients even when major changes in renal function occurred.

The cost effectiveness of this TDM intervention was not formally addressed. Previous reports have failed to prove unequivocal benefit due to the complexity of the analysis required. The major difficulty is accurate measurement of the value of benefit derived from more effective treatment and decreased toxicity. The additional costs of use of the OPT program in this study were minimal and no additional infrastructure was required. The OPT program is available from the Department of Pharmacology, amikacin assays are done routinely in the Department of Microbiology and twice weekly aminoglycoside assay is a condition of drug use in the Department of Haematology treatment protocol. The Unit pharmacist can easily be trained to do the kinetic predictions which take less than 10 minutes per patient for each entry. Thus the only additional cost incurred is that of the two additional drug assays after the first dose. On the basis of both published results and the findings of the survey conducted at GSH in 1990 (Groenewald P. et al personal communication) it would appear that measurement of aminoglycoside levels without use of a predictive model to optimally

utilize results is not cost effective. The use of a predictive TDM method would thus seem rational.

6. CONCLUSION.

A Bayesian Computer based TDM program was introduced for the control of amikacin drug levels in a high risk target population. Available evidence suggests that this should improve therapeutic efficacy and reduce toxicity in a cost effective manner. This study failed to establish any significant difference in aminoglycoside kinetic parameters for patients with profound neutropaenia but this finding is of limited significance because of factors related to study design and the kinetic model used. OPT yielded satisfactory but suboptimal performance accuracy which may be improved by revision of the population criteria used although this was not established in this study.

The OPT TDM method can be easily applied for aminoglycoside therapy to all ICUs at GSH and has the potential to significantly aid medical staff in resolving the recurrent problem of failure to achieve therapeutic aminoglycoside levels. Routine use of the method in all GSH ICUs is thus strongly recommended.

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8. INDEX OF ABBREVIATIONS.

ABW. - actual body weight

AUC. - area under curve of concentration time plot.

B-lactam - Beta lactam antibiotic class

Cl. - clearance

^ - logarithmic exponential

ECF. - extracellular fluid

GFR. - glomerular filtration rate

GSH. - Groote Schuur Hospital

ICU. - intensive care unit

MAE. - mean absolute error

ME. - mean error

MIC. - minimal inhibitory concentration

PAE.- post antibiotic effect

PC. - personal computer

RMSE. - root mean squared error

SZ. - Sawchuck - Zaske

t1/2 - half life

TDM. - therapeutic drug monitoring

Unit - Isolation Unit of the Department of Haematology

Vd - volume of distribution

9. APPENDIX.

Individual patient result are represented in two formats.

1) The numerical table contains the following data:

NO - the number of administered dose.

DATE - date of drug administration.

DOSE - total amikacin dose administered in mg.

mg/kg - dose administered expressed in mg /kg actual body weight.

TIME - clock time of administration.

INT - time in hours from start of administration of previous dose of amikacin.

PEAK - measured peak amikacin level in mg/l.

P/PEAK - OPT prediction of peak level (in mg/l) based on kinetic parameters estimated from information available at the time of previous drug level assay.

ACH-PRED - achieved minus predicted amikacin levels in mg/l.

TROUGH - measured trough amikacin level in mg/l.

P/TR - OPT prediction of amikacin trough level in mg/l.

TR-PRED - achieved minus predicted trough amikacin level in mg/l.

VD - amikacin volume of distribution estimate derived from the OPT program using all data at the time of estimation and expressed in litre.

CL - amikacin clearance estimate derived from OPT program and expressed in l/hr.

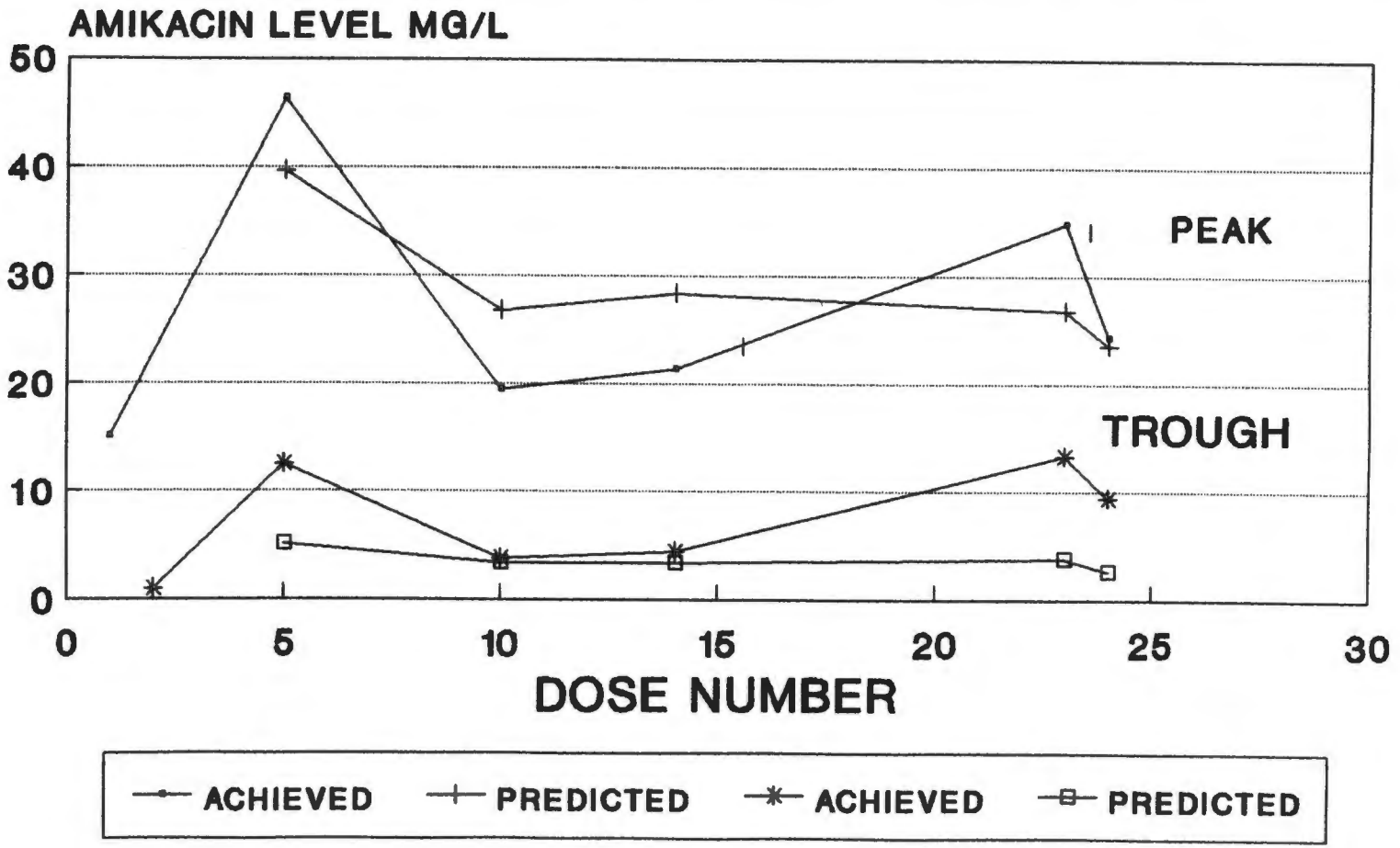
T1/2 - amikacin half life estimate derived from the OPT program and expressed in hours.

2) The graphical format displays achieved and predicted peak and trough levels on a common X axis which represents dose number. Each graph covers the period of therapy for a single patient and demonstrates visually the amikacin levels achieved as compared to those predicted.

PATIENT 1

NO	DOSE	mg/kg	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	500	9.3	0:00	15.1			1.0			20.1	5.9	5.1
2	500	9.3	11:15									
3	900	16.7	14:00									
4	900	16.7	6:00									
5	900	16.7	6:00	46.4	39.7	6.7	12.5	5.1	7.4	18.3	5.4	2.4
6	550	10.2	6:00									
7	550	10.2	8:00									
8	550	10.2	8:00									
9	550	10.2	8:00									
10	550	10.2	7:00	19.5	26.8	-7.3	3.8	3.4	0.4	20.1	6.1	2.3
11	550	10.2	9:00									
12	650	12.1	8:00									
13	650	12.1	8:00									
14	650	12.1	8:00	21.4	28.4	-7.0	4.4	3.4	1.0	22.0	6.2	2.5
15	650	12.1	8:00									
16	650	12.1	8:00									
17	650	12.1	8:00									
18	650	12.1	8:00									
19	650	12.1	8:00									
20	650	12.1	8:00									
21	650	12.1	8:00									
22	650	12.1	8:00									
23	650	12.1	8:00	34.9	22.0	12.9	13.3	4.4	8.9	23.4	3.0	5.3
24	550	10.2	18:00	24.3	23.6	0.7	9.4	2.6	6.8	36.1	2.7	9.1
Average:	640	11.9		26.9	28.1	1.2	7.4	3.8	4.9	23.3	4.9	4.5

TRIAL 1
ACHIEVED VS PREDICTED LEVELS

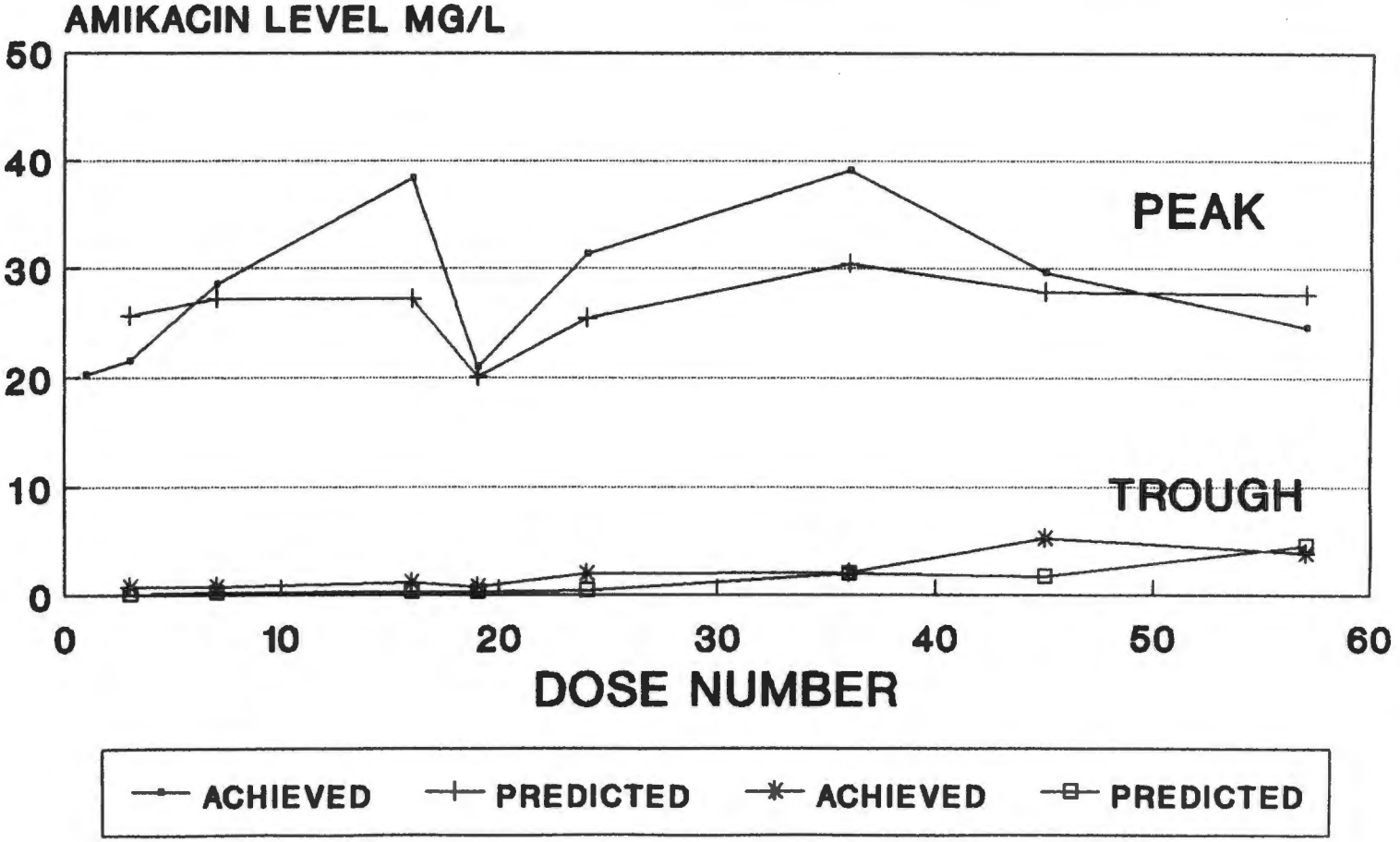


PATIENT 2

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	13/06/90	750	13.8	10:50	0:00	20.3						16.510	12.795	0.9
2	13/06/90	750	13.8	22:00	11:10									
3	14/06/90	750	13.8	5:00	7:00	21.5	25.6	-4.1	0.4	0.1	0.3	18.749	13.008	1.0
5	14/06/90	850	15.7	22:00	8:00									
6	15/06/90	850	15.7	6:00	8:00									
7	15/06/90	850	15.7	14:00	8:00	28.5	27.2	1.3	0.4	0.2	0.2	18.738	12.076	1.1
8	15/06/90	850	15.7	22:00	8:00									
9	16/06/90	850	15.7	6:00	8:00									
10	16/06/90	850	15.7	14:00	8:00									
11	16/06/90	850	15.7	22:00	8:00									
12	17/06/90	850	15.7	6:00	8:00									
13	17/06/90	850	15.7	14:00	8:00									
14	17/06/90	850	15.7	22:00	8:00									
15	18/06/90	850	15.7	6:00	8:00									
16	18/06/90	850	15.7	14:00	8:00	38.4	28.3	10.1	1.2	0.3	0.9	15.963	9.722	1.1
17	18/06/90	500	9.2	22:30	8:30									
18	19/06/90	500	9.2	6:30	8:00									
19	19/06/90	500	9.2	14:30	8:00	21.0	20.1	0.9	0.4	0.3	0.1	15.558	8.964	1.2
20	19/06/90	600	11.1	22:30	8:00									
21	20/06/90	600	11.1	6:30	8:00									
22	20/06/90	600	11.1	14:00	8:00									
23	20/06/90	600	11.1	22:30	8:00									
24	21/06/90	600	11.1	6:30	8:00	31.3	25.4	5.9	2.0	0.4	1.6	15.477	6.010	1.8
25	21/06/90	600	11.1	14:30	8:00									
26	21/06/90	600	11.1	22:30	8:00									
27	22/06/90	600	11.1	6:30	8:00									
28	22/06/90	600	11.1	14:30	8:00									
29	22/06/90	600	11.1	22:30	8:00									
30	23/06/90	600	11.1	6:30	8:00									
31	23/06/90	600	11.1	14:30	8:00									
32	23/06/90	600	11.1	22:30	8:00									
33	24/06/90	600	11.1	6:30	8:00									
34	24/06/90	600	11.1	14:30	8:00									
35	24/06/90	600	11.1	22:30	8:00									
36	25/06/90	600	11.1	6:30	8:00	39.1	30.4	8.7	2.1	2.0	0.1	13.983	5.533	1.8
37	25/06/90	500	9.2	14:30	8:00									
38	25/06/90	500	9.2	22:30	8:00									
39	26/06/90	500	9.2	6:30	8:00									
40	26/06/90	500	9.2	14:30	8:00									
41	26/06/90	500	9.2	22:30	8:00									
42	27/06/90	500	9.2	6:30	8:00									
43	27/06/90	500	9.2	14:30	8:00									
44	27/06/90	500	9.2	22:30	8:00									
45	28/06/90	500	9.2	6:30	8:00	29.6	27.8	1.8	5.2	1.7	3.5	15.413	3.957	2.7
46	28/06/90	500	9.2	14:30	8:00									
47	28/06/90	500	9.2	22:30	8:00									
48	29/06/90	500	9.2	6:30	8:00									
49	29/06/90	450	8.3	14:30	8:00									
50	29/06/90	450	8.3	22:30	8:00									
51	30/06/90	450	8.3	6:30	8:00									
52	30/06/90	450	8.3	14:30	8:00									
53	30/06/90	450	8.3	22:30	8:00									
54	01/07/90	450	8.3	6:30	8:00									
55	01/07/90	450	8.3	14:30	8:00									
56	01/07/90	450	8.3	22:30	8:00									
57	02/07/90	450	8.3	6:30	8:00	24.6	27.6	-3.0	3.9	4.6	-0.7	15.614	4.400	2.5
Average:		611	11.3			28.3	26.6	2.7	2.0	1.2	0.8	16.223	8.496	1.6

TRIAL 2

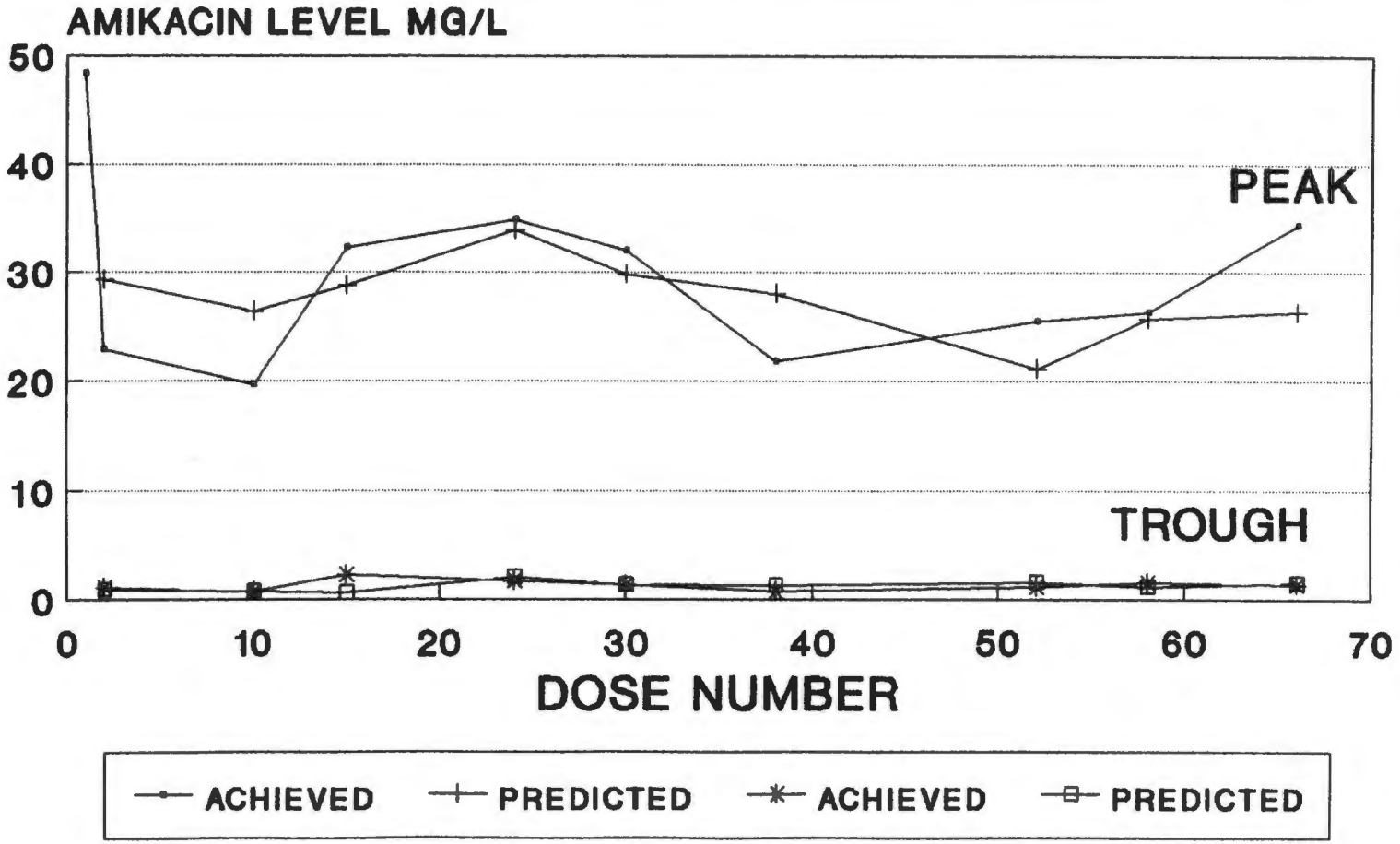
ACHIEVED VS PREDICTED LEVELS



PATIENT 3

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	20/06/90	1000	17.8	18:40	0:00	48.4						13.735	4.400	2.2
2	21/06/90	500	8.9	8:00	13:20	22.9	29.3	-6.4	1.1	0.9	0.2	15.366	4.803	2.2
3	21/06/90	500	8.9	20:00	12:00									
4	22/06/90	500	8.9	8:00	12:00									
5	22/06/90	500	8.9	20:00	12:00									
6	23/06/90	500	8.9	8:00	12:00									
7	23/06/90	500	8.9	20:00	12:00									
8	24/06/90	500	8.9	8:00	12:00									
9	24/06/90	500	8.9	20:00	12:00									
10	25/06/90	500	8.9	8:00	12:00	19.7	26.4	-6.7	0.4	0.8	-0.4	16.314	5.700	2.0
11	25/06/90	600	10.7	20:00	12:00									
12	26/06/90	600	10.7	8:00	12:00									
13	26/06/90	600	10.7	20:00	12:00									
14	26/06/90	600	10.7	8:00	12:00									
15	27/06/90	600	10.7	22:00	12:00									
16	28/06/90	600	10.7	6:30	10:30	32.3	28.8	3.5	2.3	0.6	1.7	14.110	4.635	2.1
17	28/06/90	600	10.7	20:00	13:30									
18	29/06/90	600	10.7	8:00	12:00									
19	29/06/90	600	10.7	20:00	12:00									
20	30/06/90	600	10.7	8:00	12:00									
21	30/06/90	600	10.7	20:00	12:00									
22	01/06/90	600	10.7	8:00	12:00									
23	01/06/90	600	10.7	20:00	12:00									
24	02/06/90	600	10.7	8:00	12:00	34.9	33.9	1.0	1.7	0.9	0.8	14.135	3.930	2.5
25	02/06/90	550	9.8	20:00	12:00									
26	03/06/90	500	8.9	8:00	12:00									
27	03/07/90	500	8.9	20:00	12:00									
28	04/07/90	500	8.9	8:00	12:00									
29	04/07/90	500	8.9	20:00	12:00									
30	05/07/90	500	8.9	8:00	12:00	32.0	29.8	2.2	1.4	1.4	0.0	13.463	3.803	2.5
31	05/07/90	450	8.0	20:00	12:00									
32	06/07/90	450	8.0	8:00	12:00									
33	06/07/90	450	8.0	22:00	12:00									
34	07/07/90	450	8.0	8:00	12:00									
35	07/07/90	450	8.0	20:00	12:00									
36	08/07/90	450	8.0	8:00	12:00									
37	08/07/90	450	8.0	20:00	12:00									
38	09/07/90	450	8.0	8:00	12:00	21.8	28.0	-6.2	0.4	1.3	-0.9	14.314	5.651	1.8
39	09/07/90	450	8.0	20:00	12:00									
40	10/07/90	450	8.0	8:00	12:00									
41	10/07/90	450	8.0	20:00	12:00									
42	11/07/90	450	8.0	8:00	12:00									
43	11/07/90	450	8.0	20:00	12:00									
44	12/07/90	450	8.0	8:00	12:00	18.4	23.6	-5.2	1.6	0.3	1.3	18.982	4.501	2.9
45	12/07/90	500	8.9	20:00	12:00									
46	13/07/90	500	8.9	8:00	12:00									
47	13/07/90	500	8.9	20:00	12:00									
48	14/07/90	500	8.9	8:00	12:00									
49	14/07/90	500	8.9	20:00	12:00									
50	15/07/90	500	8.9	8:00	12:00									
51	15/07/90	500	8.9	20:00	12:00									
52	16/07/90	500	8.9	8:00	12:00	25.5	21.1	4.4	1.2	1.6	-0.4	16.395	4.548	2.5
53	16/07/90	500	8.9	20:00	12:00									
54	17/07/90	500	8.9	8:00	12:00									
55	17/07/90	500	8.9	20:00	12:00									
56	18/07/90	500	8.9	8:00	12:00									
57	18/07/90	500	8.9	20:00	12:00									
58	19/07/90	500	8.9	8:00	12:00	26.3	25.7	0.6	1.6	1.2	0.4	16.031	4.230	2.7
59	19/07/90	500	8.9	20:00	12:00									
60	20/07/90	500	8.9	8:00	12:00									
Average:		521	9.3			28.2	27.4	-1.4	1.3	1.0	0.3	15.285	4.620	2.3

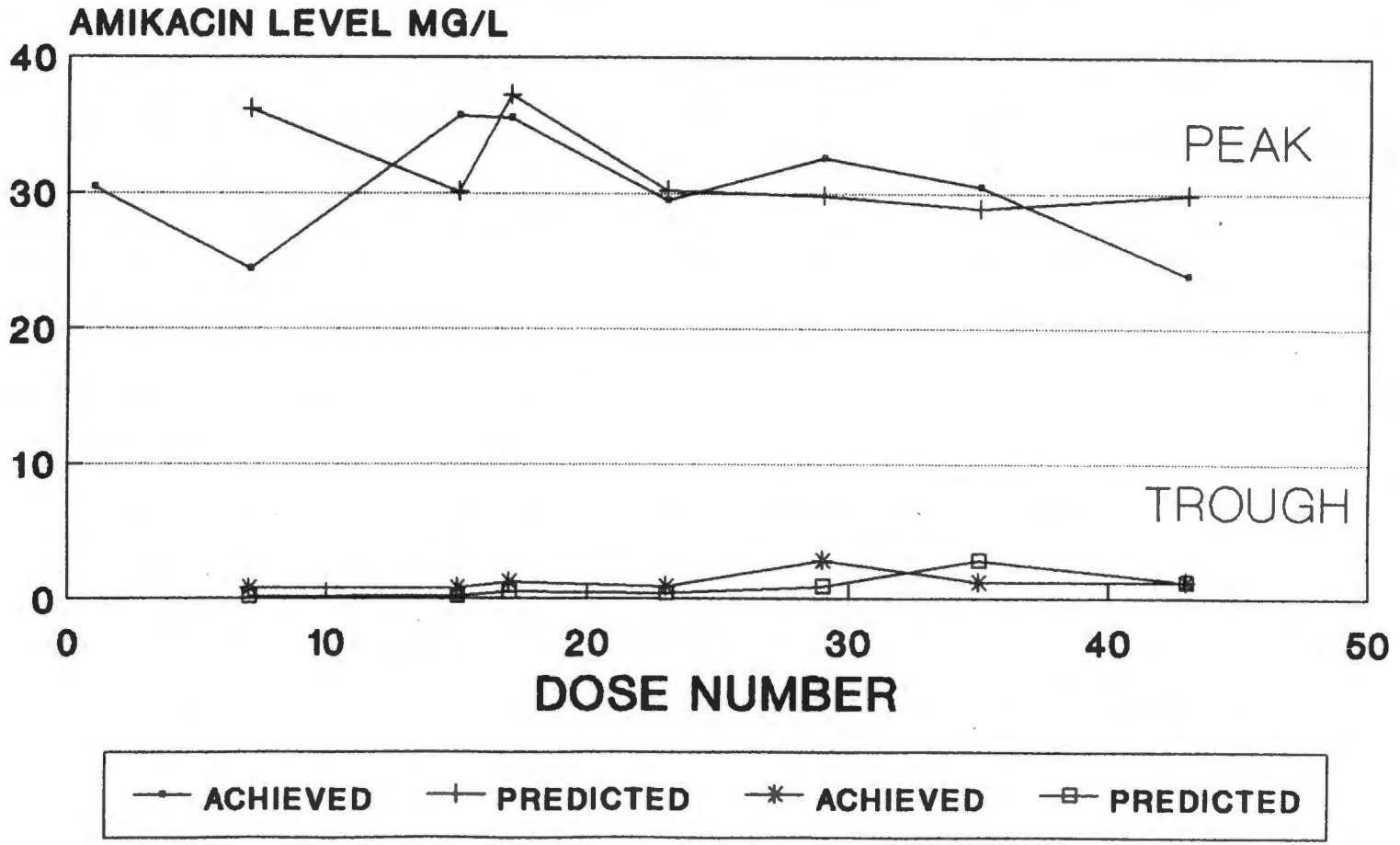
TRIAL 3
ACHIEVED VS PREDICTED LEVELS



PATIENT 4

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	25/06/90	750	14.1	10:10	0:00	30.4						13.785	7.581	1.3
2	25/06/90	750	14.1	20:00	9:50									
3	26/06/90	750	14.1	8:00	12:00									
4	26/06/90	750	14.1	22:00	12:00									
5	27/06/90	750	14.1	8:00	12:00									
6	27/06/90	750	14.1	20:00	12:00									
7	28/06/90	750	14.1	6:30	10:30	24.4	36.2	-11.8	0.4	0.1	0.3	18.128	7.897	1.6
8	28/06/90	750	14.1	20:00	13:30									
9	29/06/90	750	14.1	8:00	12:00									
10	29/06/90	750	14.1	20:00	12:00									
11	30/06/90	750	14.1	8:00	12:00									
12	30/06/90	750	14.1	20:00	12:00									
13	01/07/90	750	14.1	8:00	12:00									
14	01/07/90	750	14.1	20:00	12:00									
15	02/07/90	750	14.1	8:00	12:00	35.7	30.1	5.6	0.4	0.2	0.2	15.088	6.044	1.7
16	02/07/90	750	14.1	20:00	12:00									
17	03/07/90	750	14.1	6:30	10:30	35.5	37.2	-1.7	1.2	0.5	0.7	15.019	5.808	1.8
18	03/07/90	600	11.3	20:00	13:30									
19	04/07/90	600	11.3	8:00	12:00									
20	04/07/90	600	11.3	20:00	12:00									
21	05/07/90	600	11.3	8:00	12:00				1.4	0.4	1.0			
22	05/07/90	550	10.3	20:00	12:00									
23	06/07/90	550	10.3	8:00	12:00	29.5	30.2	-0.7	0.9	0.4	0.5	14.859	4.734	2.2
24	06/07/90	550	10.3	20:00	12:00									
25	07/07/90	550	10.3	8:00	12:00									
26	07/07/90	550	10.3	20:00	12:00									
27	08/07/90	550	10.3	8:00	12:00									
28	08/07/90	550	10.3	20:00	12:00									
29	09/07/90	550	10.3	8:00	12:00	32.6	29.8	2.8	2.8	0.9	1.9	14.552	4.077	2.5
30	09/07/90	500	9.4	20:00	12:00									
31	10/07/90	500	9.4	8:00	12:00									
32	10/07/90	500	9.4	20:00	12:00									
33	11/07/90	500	9.4	8:00	12:00									
34	11/07/90	500	9.4	20:00	12:00									
35	12/07/90	500	9.4	8:00	12:00	30.5	28.9	1.6	1.2	1.3	-0.1	13.881	4.018	2.4
36	12/07/90	500	9.4	20:00	12:00									
37	13/07/90	500	9.4	8:00	12:00									
38	13/07/90	500	9.4	20:00	12:00									
39	14/07/90	500	9.4	8:00	12:00									
40	14/07/90	500	9.4	20:00	12:00									
41	15/07/90	500	9.4	8:00	12:00									
42	15/07/90	500	9.4	20:00	12:00									
43	16/07/90	500	9.4	8:00	12:00	23.9	29.9	-6.0	1.2	1.2	0.0	16.211	4.538	2.5
44	16/07/90	500	9.4	20:00	12:00									
45	17/07/90	500	9.4	8:00	12:00									
Average:		612	11.5			30.3	31.8	-1.5	1.2	0.6	0.6	15.190	5.587	2.0

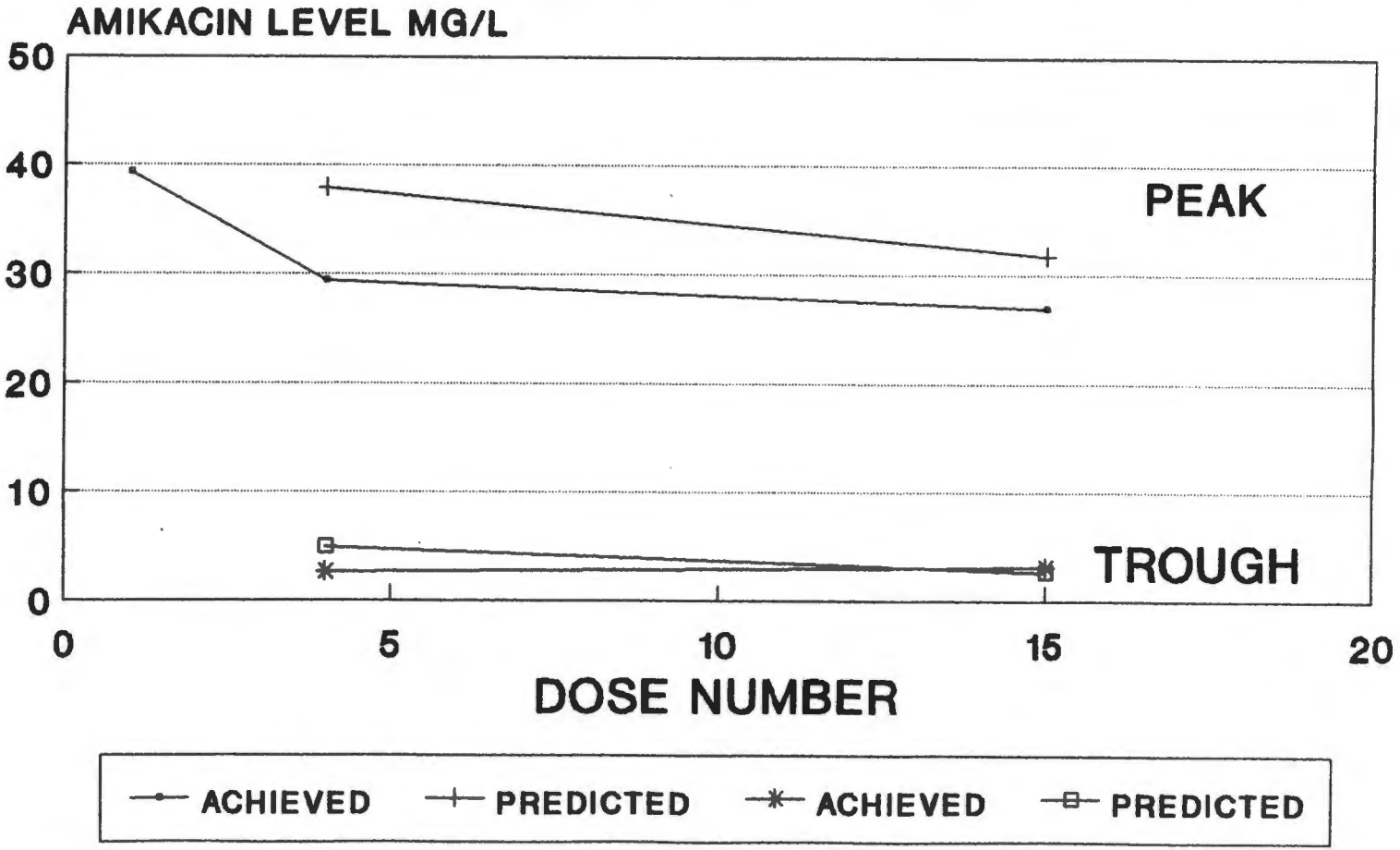
TRIAL 4
ACHIEVED VS PREDICTED LEVELS



PATIENT 5

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	27/06/90	750	12.2	12:30	0:00	39.3						12.915	3.778	3.9
2	27/06/90	550	8.9	22:30	10:00									
3	28/06/90	550	8.9	6:30	8:00									
4	28/06/90	550	8.9	14:30	8:00	29.4	37.9	-8.5	2.6	4.9	-2.3	14.127	5.020	2.0
5	28/06/90	550	8.9	22:30	8:00									
6	29/06/90	550	8.9	6:30	8:00									
7	29/06/90	550	8.9	14:30	8:00									
8	29/06/90	550	8.9	22:30	8:00									
9	30/06/90	550	8.9	6:30	8:00									
10	30/06/90	550	8.9	14:30	8:00									
11	30/06/90	550	8.9	22:30	8:00									
12	01/07/90	550	8.9	6:30	8:00									
13	01/07/90	550	8.9	14:30	8:00									
14	01/07/90	550	8.9	22:30	8:00									
15	02/07/90	550	8.9	6:30	8:00	26.9	31.7	-4.8	3.1	2.6	0.5	15.414	5.088	2.1
16	02/07/90	550	8.9	14:30	8:00									
17	02/07/90	550	8.9	22:30	8:00									
Average:		562	9.1			31.9	34.8	-6.7	2.9	3.8	-0.9	14.152	4.629	2.7

TRIAL 5
ACHIEVED VS PREDICTED LEVELS

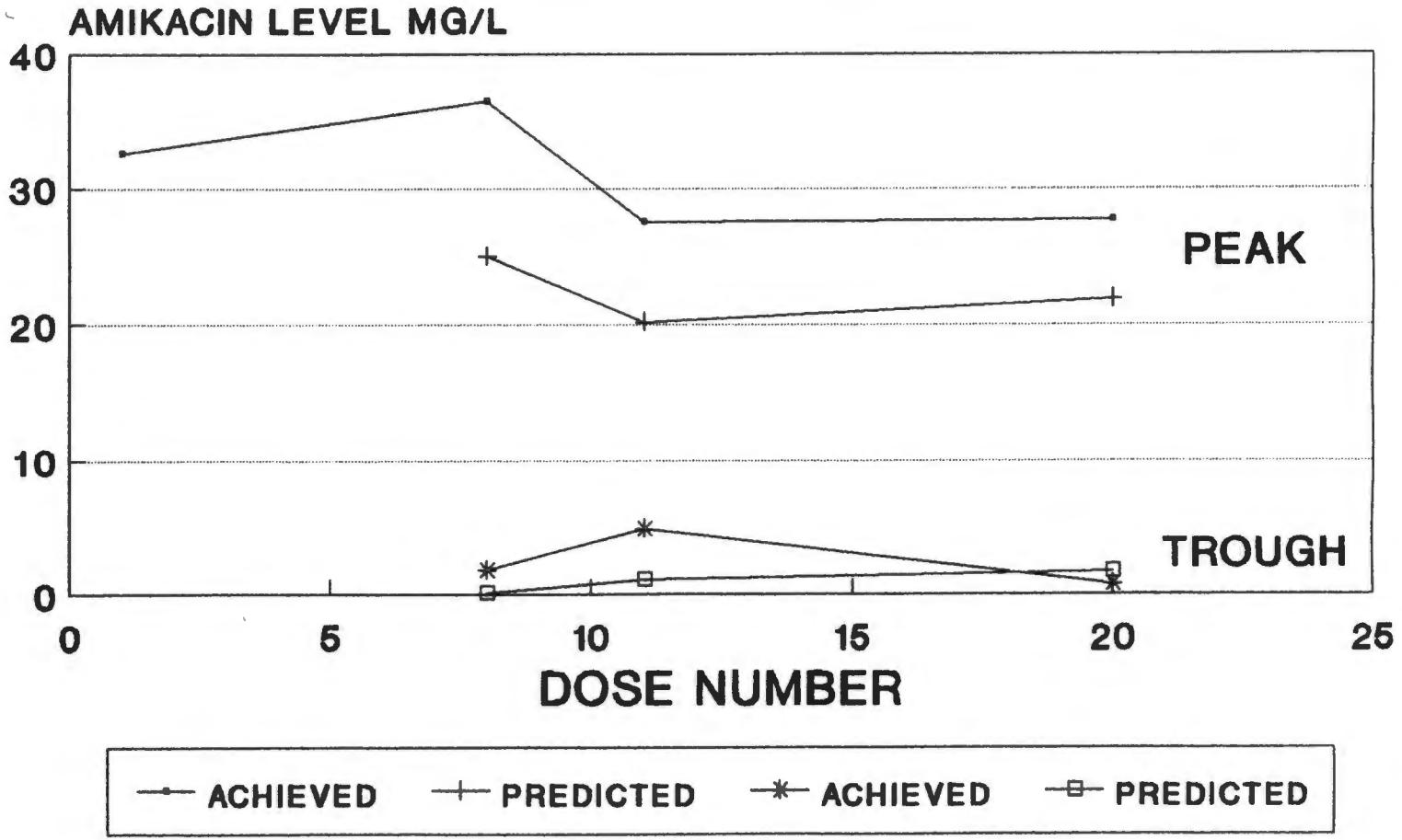


PATIENT 6

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1														
	02/07/90	750	13.1	23:30	0:00	32.6						11.555	10.029	0.8
2	03/07/90	500	8.7	8:00	8:30									
3	03/07/90	550	9.6	16:30	8:30									
4	03/07/90	550	9.6	22:30	8:00									
5	04/07/90	550	9.6	6:30	8:00									
6	04/07/90	550	9.6	14:30	8:00									
7	04/07/90	550	9.6	22:30	8:00									
8	05/07/90	550	9.6	6:30	8:00	36.5	25.0	11.5	1.8	0.1	1.7	18.734	7.904	1.6
9	05/07/90	550	9.6	14:30	8:00									
10	05/07/90	500	8.7	22:30	8:00									
11	06/07/90	500	8.7	6:30	8:00	27.5	20.2	7.3	4.9	1.1	3.8	18.297	6.736	1.9
12	06/07/90	500	8.7	14:30	8:00									
13	06/07/90	500	8.7	22:30	8:00									
14	07/07/90	500	8.7	6:30	8:00									
15	07/07/90	500	8.7	14:30	8:00									
16	07/07/90	500	8.7	22:30	8:00									
17	08/07/90	500	8.7	6:30	8:00									
18	08/07/90	500	8.7	14:30	8:00									
19	08/07/90	500	8.7	22:30	8:00									
20	09/07/90	500	8.7	6:30	8:00	27.7	21.9	5.8	0.4	1.7	-1.3	12.260	6.889	1.2
21	09/07/90	500	8.7	14:30	8:00									
22	09/07/90	500	8.7	22:30	8:00									
23	10/07/90	500	8.7	6:30	8:00									
24	10/07/90	500	8.7	14:30	8:00									
25	10/07/90	500	8.7	22:30	8:00									
Average:		524	9.1			31.1	22.4	8.2	2.4	1.0	1.4	15.212	7.890	1.4

TRIAL 6

ACHIEVED VS PREDICTED LEVELS

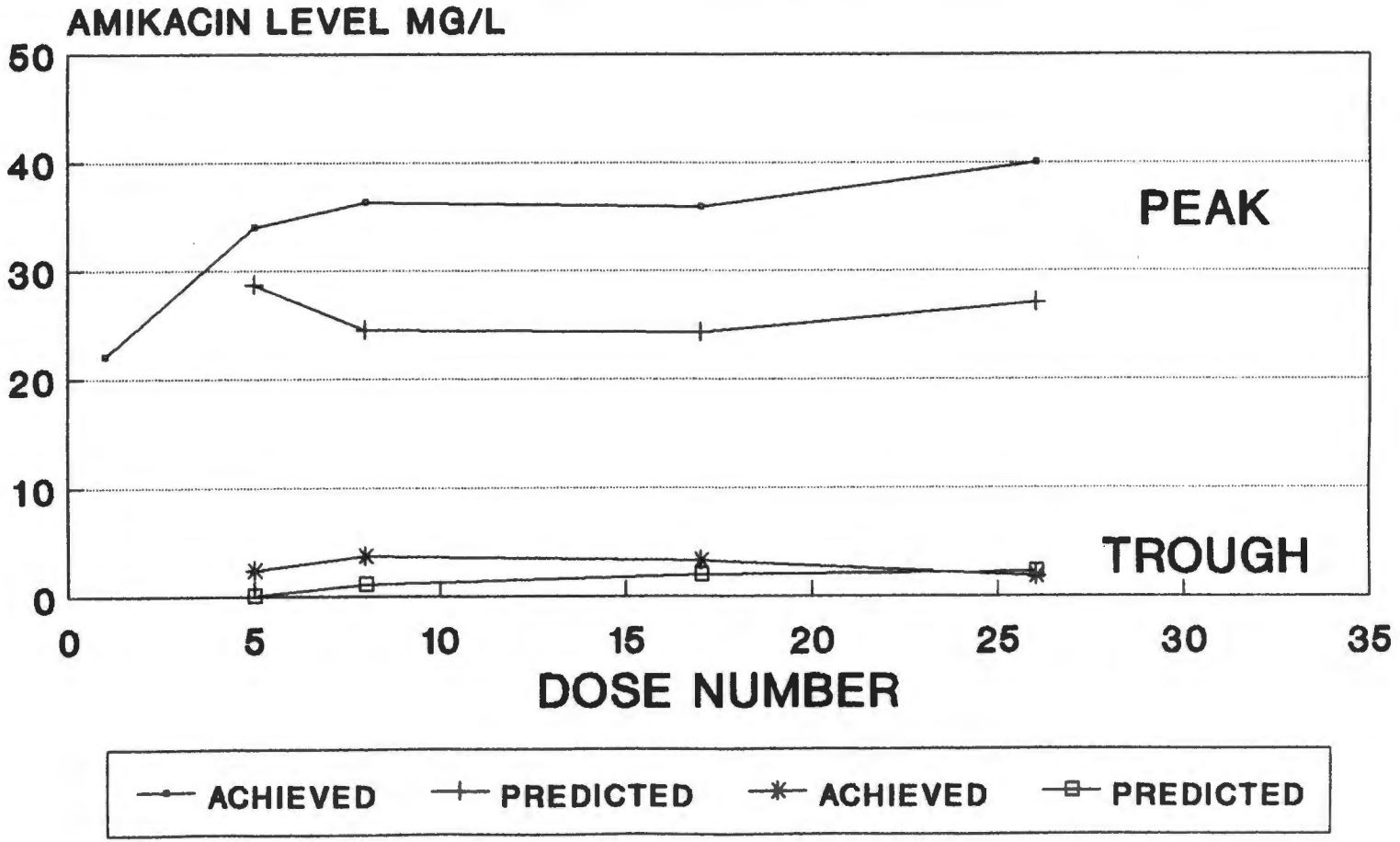


PATIENT 7

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	03/07/90	750	13.0	18:30	0:00	22.1						14.991	12.588	0.8
2	04/07/90	500	13.0	8:00	13:30									
3	04/07/90	800	13.0	14:30	6:30									
4	04/07/90	800	13.0	22:30	8:00									
5	05/07/90	800	13.9	6:30	8:00	34.0	28.7	5.3	2.4	0.1	2.3	22.591	10.100	1.6
6	05/07/90	800	13.9	14:30	8:00									
7	05/07/90	750	13.0	22:30	8:00									
8	06/07/90	750	13.0	6:30	8:00									
9	06/07/90	750	13.0	14:30	8:00	36.3	24.5	11.8	3.7	1.1	2.6	23.475	8.315	2.0
10	06/07/90	700	12.2	22:30	8:00									
11	07/07/90	700	12.2	6:30	8:00									
12	07/07/90	700	12.2	14:30	8:00									
13	07/07/90	700	12.2	22:30	8:00									
14	08/07/90	700	12.2	6:30	8:00									
15	08/07/90	700	12.2	14:30	8:00									
16	08/07/90	700	12.2	22:30	8:00									
17	09/07/90	700	12.2	6:30	8:00	35.9	24.3	11.6	3.3	2.0	1.3	18.080	6.362	2.0
18	09/07/90	700	12.2	14:30	8:00									
19	09/07/90	600	10.4	22:30	8:00									
20	10/07/90	600	10.4	6:30	8:00									
21	10/07/90	600	10.4	14:30	8:00									
22	10/07/90	600	10.4	22:00	8:00									
23	11/07/90	600	10.4	6:30	8:00									
24	11/07/90	600	10.4	14:30	8:00									
25	11/07/90	600	10.4	22:30	8:00									
26	12/07/90	600	10.4	6:30	8:00	40.0	27.1	12.9	1.9	2.3	-0.4	14.983	5.972	1.7
27	12/07/90	500	8.7	14:30	8:00									
28	12/07/90	500	8.7	22:30	8:00									
29	13/07/90	500	8.7	6:30	8:00									
Average:		666	11.7			33.7	26.2	10.4	2.8	1.4	1.5	18.824	8.667	1.6

TRIAL 7

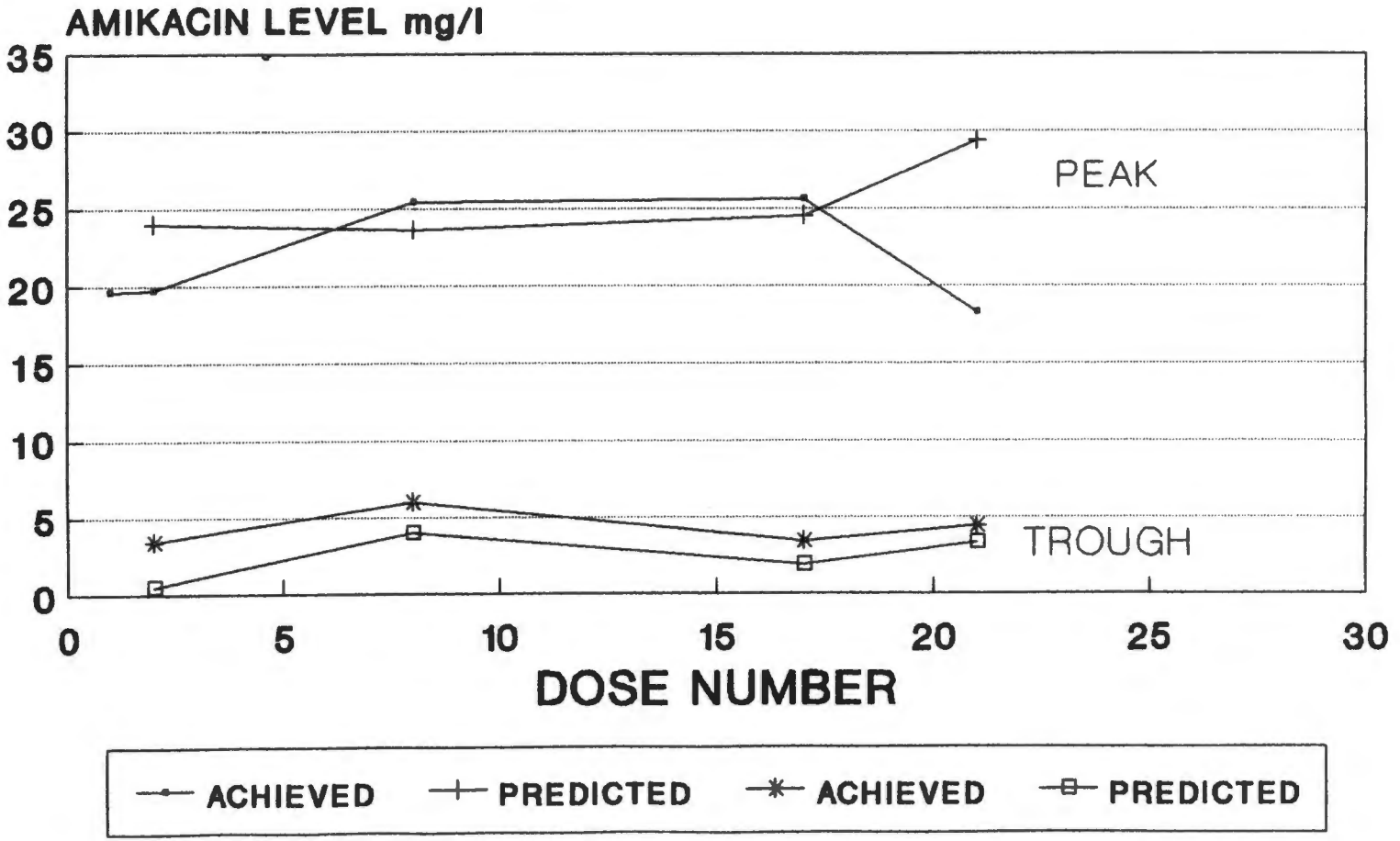
ACHIEVED VS PREDICTED LEVELS



PATIENT 8

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	22/07/90	500	8.6	19:20	0:00	19.6						16.228	5.719	2.0
2	23/07/90	500	8.6	8:00	12:40	19.7	24.0	-4.3	3.4	0.5	2.9	21.958	3.530	4.3
3	23/07/90	500	8.6	20:00	12:00									
4	24/07/90	500	8.6	8:00	12:00									
5	24/07/90	500	8.6	20:00	12:00									
6	25/07/90	500	8.6	8:00	12:00									
7	25/07/90	500	8.6	20:00	12:00									
8	26/07/90	500	8.6	8:00	12:00	25.4	23.6	1.8	6.0	4.0	2.0	19.612	2.928	4.6
9	27/07/90	500	8.6	2:00	18:00									
10	27/07/90	500	8.6	20:00	18:00									
11	28/07/90	500	8.6	14:00	18:00									
12	29/07/90	500	8.6	14:00	18:00									
13	30/07/90	500	8.6	2:00	18:00									
14	30/07/90	500	8.6	8:00	6:00									
15	01/08/90	500	8.6	0:00	40									
16	01/08/90	500	8.6	18:00	18:00									
17	02/08/90	500	8.6	12:00	18:00	25.6	24.5	1.1	3.4	1.9	1.5	17.094	2.206	5.4
18	03/08/90	500	8.6	6:00	18:00									
19	05/08/90	500	8.6	0:00	18:00									
20	05/08/90	500	8.6	18:00	18:00									
21	06/08/90	500	8.6	12:00	18:00	18.3	29.4	-11.1	4.4	3.3	1.1	23.280	2.859	1.5
22	07/08/90	500	8.6	8:00	18:00									
Average:		500	8.6			21.7	25.4	-3.1	4.3	2.4	1.9	19.634	3.448	3.6

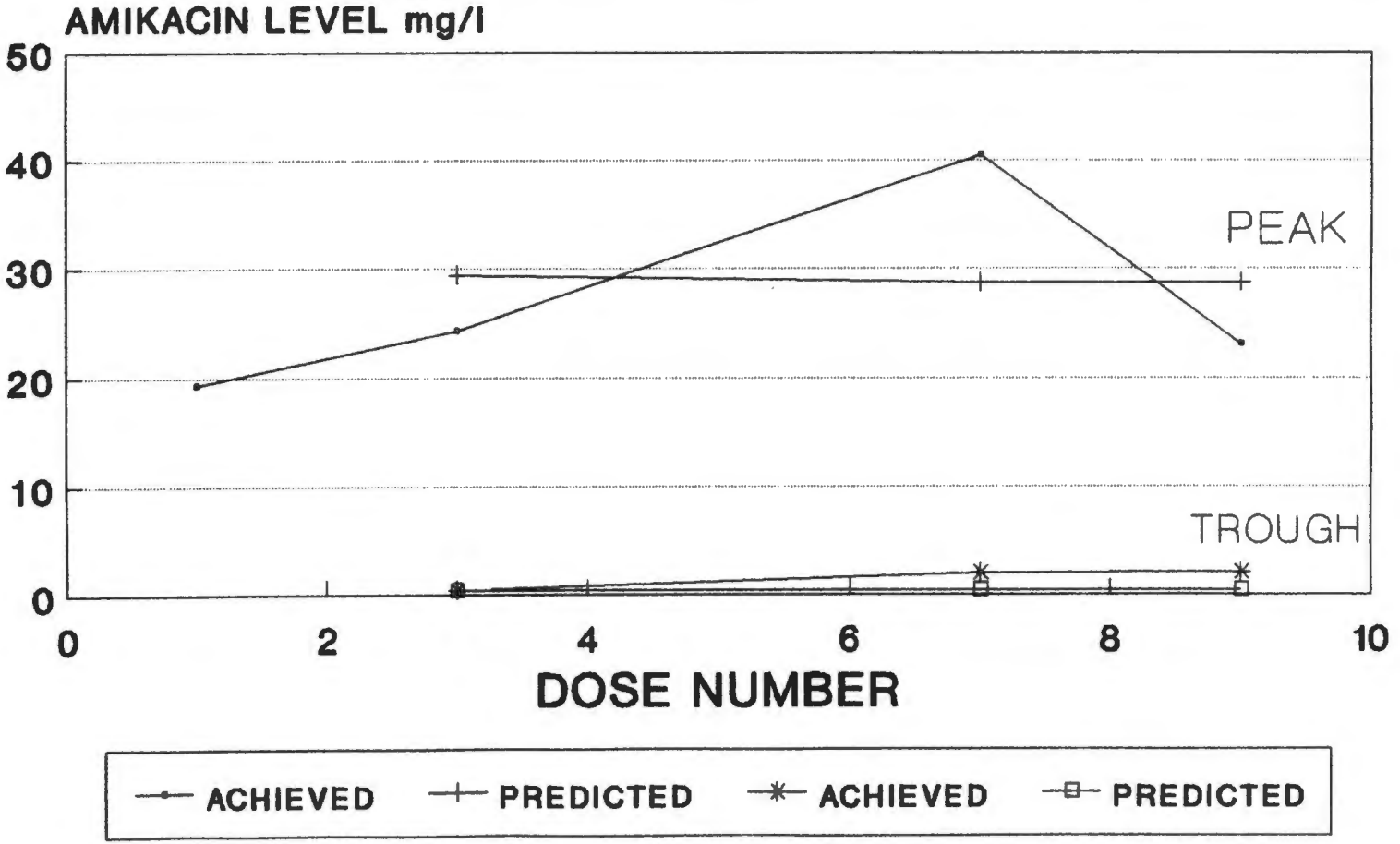
TRIAL 8
ACHIEVED VS PREDICTED



PATIENT 9

	NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
	1	30/07/90	500	9.4	17:30	0:00	19.3								
	2	30/07/90	500	9.4	22:00	2:30									
	3	31/07/90	500	9.4	8:00	12:00	24.3			0.4			15.249	6.129	1.7
	4	31/07/90	500	9.4	20:00	12:00									
	5	01/08/90	600	11.3	8:00	12:00									
	6	01/08/90	600	11.3	20:00	12:00									
	7	02/08/90	600	11.3	8:00	12:00	40.5	29.4	11.1	2.0	0.4	1.6	13.125	5.147	1.8
	8	02/08/90	500	9.4	20:00	12:00									
	9	03/08/90	500	9.4	8:00	12:00	23.1	28.7	-5.6	2.1	0.4	1.7	14.102	5.174	1.9
	10	03/08/90	500	9.4	20:00	12:00									
	11	04/08/90	500	9.4	8:00	12:00									
	12	04/08/90	500	9.4	20:00	12:00									
	13	05/08/90	500	9.4	8:00	12:00									
Average:			523	9.8			26.8	29.1	2.8	1.5	0.4	1.7	14.159	5.483	1.8

TRIAL 9
ACHIEVED VS PREDICTED



PATIENT 10

NO	DATE	DOSE	mg/kg	TIME	INT	PEAK	P/PEAK	ACH-PRED	TROUGH	P/TR	TR-PRED	VD	CL	T1/2
1	01/08/90	750	11.1	11:45	0:00	21.1						20.828	8.549	1.7
2	01/08/90	800	11.8	20:00	10:15									
3	02/08/90	800	11.8	8:00	12:00	16.3	28.5	-12.2	0.4	0.3	0.1	27.385	10.091	1.9
4	02/08/90	1000	14.8	20:00	12:00									
5	03/08/90	1000	14.8	8:00	12:00	40.2	28.1	12.1	1.5	0.5	1.0	24.739	8.840	0.5
6	03/08/90	900	13.3	20:00	12:00									
7	04/08/90	900	13.3	8:00	12:00									
8	04/08/90	900	13.3	20:00	12:00									
9	05/08/90	900	13.3	8:00	12:00									
10	05/08/90	900	13.3	20:00	12:00									
11	06/08/90	900	13.3	8:00	12:00	44.8	28.3	16.5	3.0	0.6	2.4	19.423	6.749	2.0
12	06/08/90	700	10.4	20:00	12:00									
13	07/08/90	700	10.4	8:00	12:00	28.7	28.2	0.5	3.7	0.6	3.1	22.173	4.317	3.6
14	07/08/90	700	10.4	20:00	12:00									
15	08/08/90	700	10.4	8:00	12:00									
16	08/08/90	700	10.4	20:00	12:00									
17	09/08/90	700	10.4	8:00	12:00	31.7	29.3	2.4	2.2	0.8	1.4	19.903	4.810	2.9
18	09/08/90	700	10.4	20:00	12:00									
19	10/08/90	700	10.4	8:00	12:00									
20	10/08/90	700	10.4	20:00	12:00									
21	11/08/90	700	10.4	8:00	12:00									
22	11/08/90	700	10.4	20:00	12:00									
23	12/08/90	700	10.4	8:00	12:00									
24	12/08/90	700	10.4	20:00	12:00									
25	13/08/90	700	10.4	8:00	12:00									
26	13/08/90	700	10.4	20:00	12:00									
27	14/08/90	700	10.4	8:00	12:00	31.0	31.1	-0.1	0.4	2.2	-1.8	17.550	6.871	1.8
28	14/08/90	700	10.4	20:00	12:00									
29	15/08/90	700	10.4	8:00	12:00									
30	15/08/90	700	10.4	20:00	12:00									
31	16/08/90	700	10.4	8:00	12:00	26.8	30.1	-3.3	1.2	0.4	0.8	19.113	6.776	2.0
32	16/08/90	700	10.4	20:00	12:00									
33	17/08/90	700	10.4	8:00	12:00									
Average:		762	11.3			30.1	29.1	2.3	1.8	0.8	1.0	21.389	7.125	2.1

TRIAL 10
ACHIEVED VS PREDICTSD