Chapter 5
MONITORING

LABORATORY MONITORING

A large fraction, perhaps even the majority, of clinical laboratory studies are ordered not for the purpose of diagnostic or prognostic classification but, rather, to monitor patients; that is, to determine if and by how much a study result has changed. The clinical applications of monitoring are diverse, embracing every aspect of patient management (Table 5.1). Four general categories of clinical use of monitoring studies can be identified: screening for subclinical disease, monitoring physiologic status, monitoring disease activity, and monitoring toxic and therapeutic agents. Therapeutic drug monitoring is discussed in a separate chapter.

SCREENING FOR SUBCLINICAL DISORDERS

As defined in Chapter 3, screening studies are used to detect serious, treatable disorders in persons who have the disorder but who have not manifested the condition clinically. Screening studies can be performed on a catch-as-catch-can basis, as exemplified by the practice of ordering screening studies as part of every hospital admission, or they can be performed according to a screening program designed to achieve certain performance criteria.

Screening programs have three components: (1) a definition of the population to be screened, (2) a rule indicating when to perform the first screening study on an individual, and (3) a schedule for performing subsequent screening studies. As regards the population to be screened, it is obvious that screening should be performed only on individuals who might have the disorder. For example, only women develop endometrial cancer, so screening for this cancer is performed only in women. The criteria used to define the screening population for many disorders are based upon the identification of demographic or clinical groups in which overt disease is found. This approach is improved by broadening the criteria to include groups in which subclinical disease is known to occur even if overt disease is not seen.

Table 5.1
Clinical Uses of Monitoring Studies

1. Detect the development of a disorder while the disorder is still subclinical
2. Assess changes in organ function, metabolic activity, or macro- or micronutritional status
3. Assess changes in organ function, metabolic activity, or macro- or micronutritional status in response to therapy
4. Detect changes in the level of activity of a disorder
5. Detect changes in the level of activity of a disorder in response to therapy
6. Follow the time course of intoxicants and poisons
7. Follow the time course of pharmacologic agents

Having defined the population for whom screening could be useful, the starting rule and schedule for repeat testing are selected so as to assure that the screening program has a reasonable probability of detecting the disorder in a screened individual while the disorder is in a treatable part of its subclinical phase. In attempting to achieve this performance goal, the program is constrained by many practical considerations, the two most important of which are the cost of performing the screening studies and the need for adequate specificity.

The probability of detecting a disorder in its subclinical phase depends upon many factors. These include the natural history of the subclinical phase, the diagnostic performance of the screening study as the disorder develops within its subclinical phase, and the timing of the first and subsequent repetitions of the screening study (Provok *et al.* 1981).

Consider a screening program consisting of a single study performed at some prescribed time in one's life. An example of such a design is a single-study screening for a genetic disorder in the neonatal period. For a single-study screening program, the probability of detecting subclinical disease equals the sensitivity of the screening study for the subclinical disease times the probability of being in the subclinical phase at the time the screening study is performed. The probability of being in the subclinical phase is determined by the natural history of the disorder and the sensitivity of the screening study is determined by the study's critical value. In order to assure that the study has adequate specificity, the

critical value should be set equal to the value that yields the threshold likelihood ratio for follow-up

$$\textit{threshold likelihood ratio for follow-up} = \frac{(1 - \textit{prevalence})\, P[\textit{rejection}]}{\textit{prevalence}\,(1 - P[\textit{rejection}])}$$

with the prevalence being the frequency of the subclinical disorder among members of the demographic group being screened at the time in life when the screening study is performed.

Figure 5.1 shows the details of the natural history of a hypothetical disorder. As depicted, there is a 10 year period in a person's life when the disease may begin, with the risk of contracting the disorder being constant throughout that period (top graph). Once begun, the disease lasts 1 to 5 years in its subclinical phase before becoming clinically manifest with an equal frequency of clinical appearance throughout that period (middle graph). The resulting prevalence of the subclinical and clinical phases of the disease are shown in the bottom graph. Notice that, given the simple patterns of onset and progression employed here, there is a 5 year period, 5 to 10 years, during which the prevalence of the subclinical phase is constant at its maximum value, 0.0116. Clearly, it is during this period that screening should be performed in a one-study program. So, let the time of the screening study be 5 years, just as the prevalence plateau is reached. How many of these subclinical cases will then be detected? That depends upon the diagnostic performance of the screening study which, in turn, is determined by the natural history of the marker measured by the study (Figure 5.2, top graph) and the distribution of the degree of development of the subclinical phase among the individuals with subclinical disease (Skates and Singer 1991). The marker concentration frequency distributions for disease-free individuals and individuals with subclinical disease as found in year 5 in the natural history of this hypothetical disorder are shown in the middle graph of Figure 5.2. The bottom graph shows the associated likelihood ratio plot.

If the threshold probability for rejection of the diagnosis is 0.1, the threshold likelihood ratio for screening follow-up is 9.5 and the appropriate critical value for the screening study is 77. This critical value yields a test sensitivity of 0.69 and a specificity of 0.996. Therefore, the sensitivity of the screening program, i.e., the probability of detecting the disease in the subclinical phase with a single

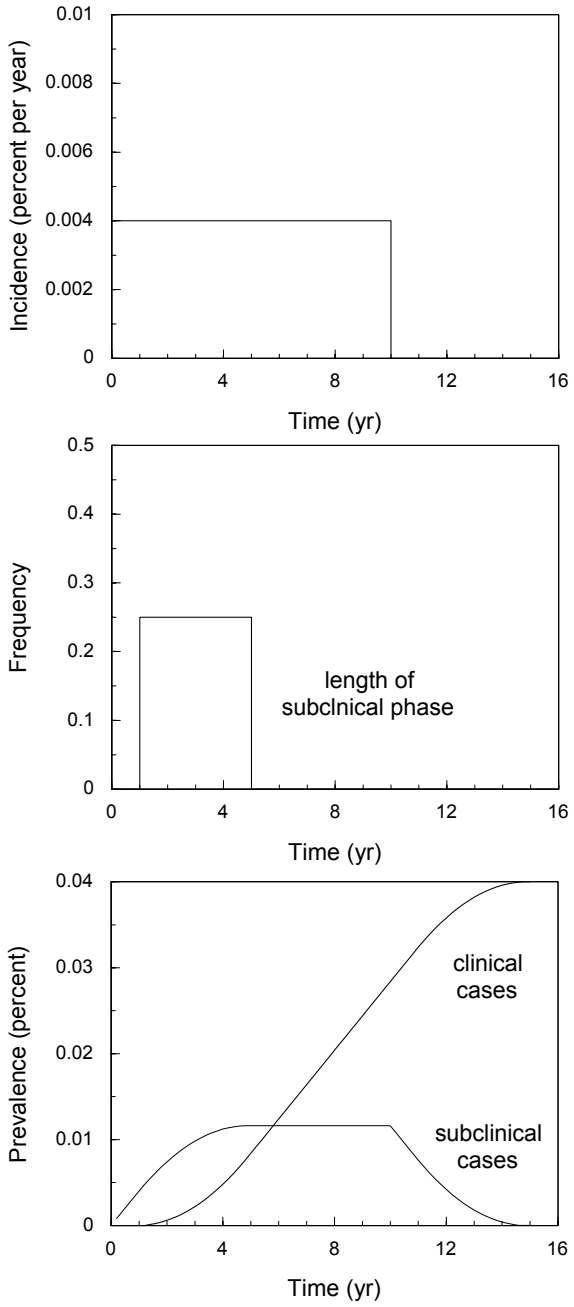

Figure 5.1 The natural history of a hypothetical disorder.

screening study, is 0.20 (the proportion of individuals with disease who are screened while in the subclinical phase, 0.29, times the single-test sensitivity of the study, 0.69). Is this expectation of detecting subclinical disease large enough to be considered reasonable? Perhaps. The expense and morbidity generated by a screening program based on this study may not be great and the benefits of identifying even 20 percent of affected individuals during the subclinical phase may be substantial. On the

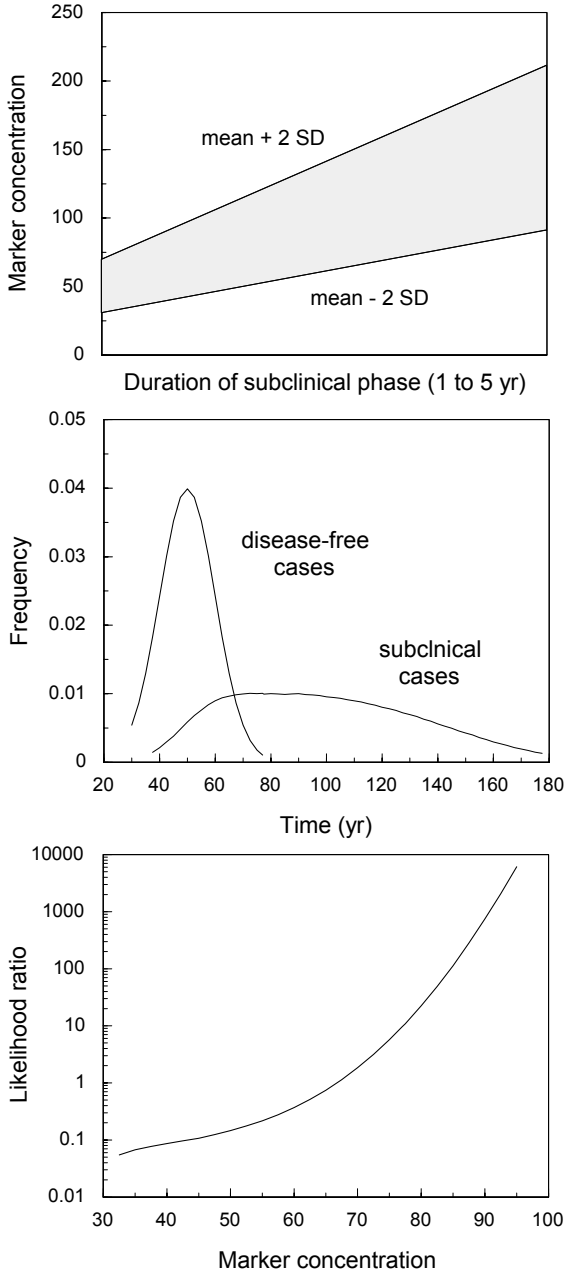

Figure 5.2 The natural history of a diagnostic marker for the hypothetical disorder (top graph). The classification characteristics of the marker at year 5 are shown in the middle and bottom graphs.

other hand, one could argue that this probability of detection is too small to justify the expense and bother of screening.

Fortunately, screening studies can usually be performed more than once. Indeed, periodic screening is the norm. In this way the probability of detecting the disease in the subclinical phase can be greatly increased (Eddy 1982). For instance, continuing the preceding example, the effect of a single screening study upon the natural history of the hypothetical disorder can be computed and the optimal timing of another study predicted. In this example, the prevalence of subclinical disease reaches a maximum 5 years following the initial screening study, after which it decreases steadily. Performing a second study in asymptomatic individuals 5 years after the first, and using the same critical value, will result in an identical impact on the disease; 69 percent of the subclinical cases will be identified and another 20 percent of cases shall have been detected while in the subclinical phase. Therefore, in this example, a second study can double the yield of the screening program. Of course, at the same time, the overall specificity of the screening program will decrease slightly. Additional increases in the number of screening studies would further increase the probability of detecting subclinical disease but at the price of reducing the specificity even more. In order to maintain an acceptable level of overall specificity for a screening program, it is necessary either to set an upper limit on the number of studies performed or to adjust the threshold probability of diagnosis to take into account the multiplicity of studies.

MONITORING PHYSIOLOGIC STATUS

The response of a clearance function marker to a sudden, persistent change in organ clearance function is illustrated in Figure 5-3. The time course of the change in functional status is shown in the upper graph. The function marker responds to the drop in its clearance rate by accumulating in the plasma until it reaches a new steady-state concentration as depicted in the lower graph. The rapidity with which the new steady state is reached depends upon the half-life of the marker; it takes 4-5 half-lives for the steady state to evolve. For example, the half-life of creatinine in the plasma in the setting of normal renal function is 4 h. At 80 percent of normal renal function, the half-life is 5 h. If renal function were to suddenly decline to 80% of normal, the plasma concentration of creatinine would increase to its new steady state value over 20-25 h (4-5 times 5 h). Notice that the time to achieve the steady state depends not upon the normal half-life of the marker substance but upon the half-life in the altered physiologic state.

Because of the presence of variability in laboratory measurement, the concentration of a marker

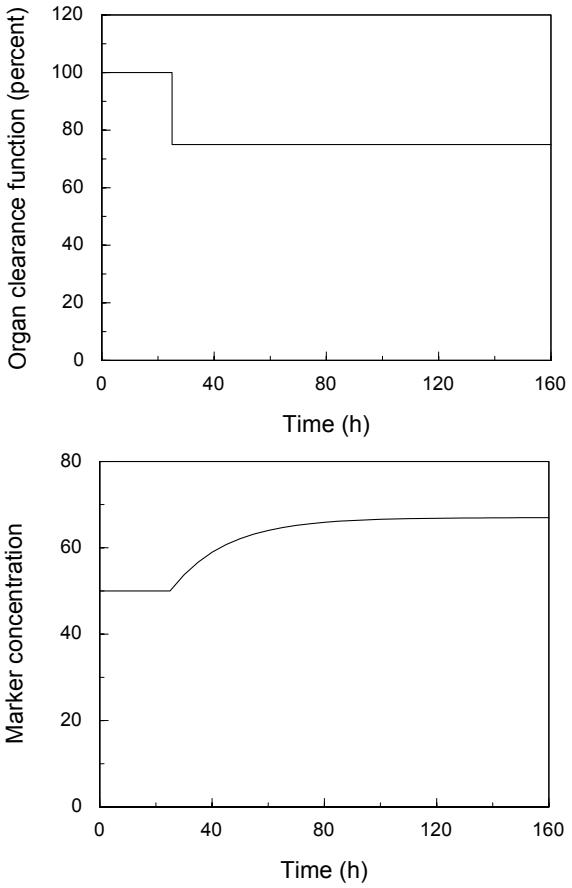

Figure 5.3 The response of a clearance function marker, lower graph, to a change in organ clearance function, upper graph.

substance must change by a certain amount before the change can be ascribed to an alteration in physiologic status rather than to measurement imprecision. In Figure 5.4, the time course of plasma marker concentrations, with and without a change in organ function, are shown as bands of values that encompass 95 percent of the concentrations that would be seen given a coefficient of variability of 10% for the measurement variability in both settings. There is overlap of the bands for a period of time after the change in organ function, indicating that during this period it is difficult to detect the change in marker concentration; the rising values can be ascribed to measurement variability. Later on, the bands are well separated, indicating that the change in marker concentration can be distinguished from the pre-existing concentration.

The length of time it takes for a ongoing change in marker concentration to be distinguishable from the preceding steady-state concentration depends upon (1) the half-life of the marker substance, (2) the magnitude of the change in physiologic status, and (3) the magnitude of the measurement variability. A short marker half-life, a large alteration in physiologic status, and a small degree of measurement variability all allow for rapid appreciation of a change in marker concentration. Conversely, a long time will be required to detect a change in the marker concentration if the marker has a long half-life, if the change in physiologic status is small, or if the measurement variability is large.

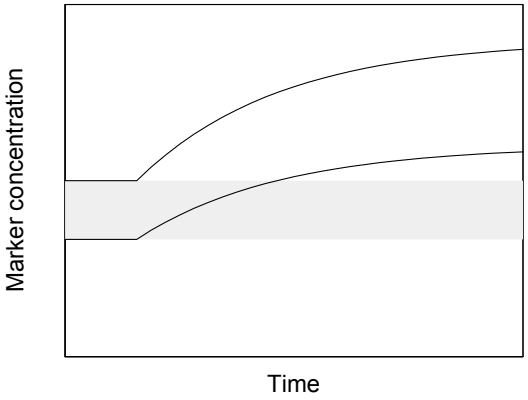

Figure 5.4 The response of an organ function marker to a change in organ function when there is variability in the measurement of the marker.

In order to provide timely insight into a patient's physiologic status, marker substances used for monitoring purposes are chosen on the basis of rapid changes in their plasma concentration following a status change. These early indicators are not always the same markers used to evaluate physiologic status in the steady state. For example, albumin, which is a standard marker of hepatic synthetic function, has a low clearance rate and, consequently, a very long half-life. Its plasma concentration changes very slowly following an alteration in synthetic function. Even following nearly complete hepatic shutdown, as occurs in fulminant hepatic necrosis, the plasma albumin concentration, corrected for changes in plasma volume, declines gradually over a period of weeks. In contrast, prealbumin, which has a short plasma half-life, shows a marked reduction in its plasma concentration in a matter of days following the onset of hepatic failure.

Measurement variability

The simplest situation encountered in physiologic monitoring is that of two study results separated in time with the clinical question being, "Has the study value changed?" Due to measurement

variability, two results will rarely be the same, even if the patient's physiologic status is unchanged. The task is to decide if the difference in the results is more likely due to measurement variability or to a change in physiologic status.

In the absence of a change in physiologic function, the study results in an individual are distributed about some mean value, avg_{indiv}, with the variability characterized by the within-individual variance, var_{indiv} which is the sum of within-individual biologic variance, $var_{biologic}$, and analytic variance, $var_{analytic}$. The mean value for the difference between two measurements is zero and the variance of the difference is

$$2\ var_{indiv}$$

If the specificity for detecting a change in concentration is set at 0.95, a significantly different value is one outside the central 95 percent range of the distribution of result differences,

$$significant\ difference \geq 2\sqrt{2\ var_{indiv}}$$

Thus, if var_{indiv} is 50 $\mu mol^2/L^2$, for example, an absolute difference in result values of 20 μmol/L or more is considered significant, at the stipulated level of specificity, and implies a change in physiologic status.

The formula is valid only if the time interval between measurements is long (Harris and Yasaka 1983) and, for some analytes, only if the interval is very long (Queraltó *et al.* 1993). At shorter intervals the within-individual biologic component of variability drops out because, for most marker substances, only small changes in concentration normally take place over a few hours. Consider, for example, the blood concentration of neutrophils. From day to day the concentration can vary as much as $3x10^9$ cells/L around an average value of $3.5x10^9$ cells/L. Over a period of 2 to 3 hours, though, the variability is probably only one-third as great. Consequently, when measurements are taken at intervals of less than 3 hours, cell concentration will show much less variability than when measurements are made daily. The variability that is present will be largely analytic.

The degree to which consecutive measurements are similar due to the sluggishness of biologic change is embodied in the statistical index, serial correlation. A serial correlation of one denotes absolute immobility of the measurement such that each measurement is identical to its predecessor. A value of zero indicates that the biologic change is rapid enough that, at the sampling interval specified, each measurement appears unrelated to those preceding it. Incorporating the idea of serial correlation, the variance of the difference between two measurements is,

$$2\ (1-\rho(\tau))\ var_{biologic} + 2\ var_{analytic}$$

where $\rho(\tau)$ is the serial correlation for the time interval τ.

For most purposes only the two extreme values for the serial correlation need to be considered. Either the sampling interval is long relative to the time needed for a biologic change and $\rho(\tau)$ is equal to zero, so that,

$$significant\ difference \geq 2\sqrt{2\ var_{indiv}}$$

or the interval is short with $\rho(\tau)$ equal to one, in which case,

$$significant\ difference \geq 2\sqrt{2\ var_{analytic}}$$

To use these formulas, estimates of var_{indiv} or $var_{analytic}$ are needed. Ideally, these estimates should be specific for the patient being studied and for the laboratory in which the analyte is measured. When there is no antecedent history of study results for the patient on which to base an estimate of var_{indiv}, the typical value of var_{indiv} as found in the medical literature can be used in its stead. Laboratory staff should be able to provide values for $var_{analytic}$ for the analytes measured in their laboratory.

For sampling intervals of intermediate length, serial correlation should be included in the calculation of the value of a significant difference,

$$significant\ difference \geq 2\sqrt{2\,(1-\rho(\tau))\,var_{biologic} + 2\,var_{analytic}}$$

remembering that $\rho(\tau)$ refers to the serial correlation of the biologic component of the measurement variance. Almost always, the form of serial correlation for which a value is readily available is the total serial correlation of repeated measurements, $r(\tau)$. This statistical parameter depends upon both the biologic and analytic variability according to the formula (Harris 1983),

$$r(\tau) = \rho(\tau)\,\frac{var_{indiv} - var_{indiv}}{var_{indiv}}$$

Substitution of this expression into the preceding formula and rearrangement shows that when using total serial correlation,

$$significant\ difference \geq 2\sqrt{2(1-r(\tau))\,var_{indiv}} \quad .$$

The next clinical situation to consider is one in which there are a number of study results for a patient during a period in which the patient's physiologic status is believed to have been constant. If there are six or more previous results, var_{indiv} can be calculated. The reason that at least six measurements are needed is that estimates based on fewer measurements have a high degree of uncertainty. This is illustrated in the lower graph in Figure 5.5. The 5th and 95th percentile confidence limits for estimates of the mean and the variance are shown. For fewer than six measurements, the 90 percent confidence interval for the variance is large, having a width more than five times greater than the calculated variance. Notice that, with six or more previous study results, avg_{indiv} can also be estimated with acceptable certainty, the 90 percent confidence interval being about 1.65 standard deviations in width.

Given a reliable estimate of var_{indiv}, the formula for a significant difference between study results can be individualized when it is used to evaluate subsequent results. However, a more sensitive way to detect a change in marker concentration is to compare subsequent study results with the distribution of results in the patient as based upon estimates of avg_{indiv} and var_{indiv}. Setting the specificity at 0.95, a change in concentration is indicated if a result is outside the central 95 percent range of preceding marker concentrations,

$$significant\ result \geq avg_{indiv} + 2\sqrt{var_{indiv}}$$

$$or \quad \leq avg_{indiv} - 2\sqrt{var_{indiv}}$$

This approach is more sensitive because it incorporates more of the prior information known about the patient—avg_{indiv} as well as var_{indiv} .

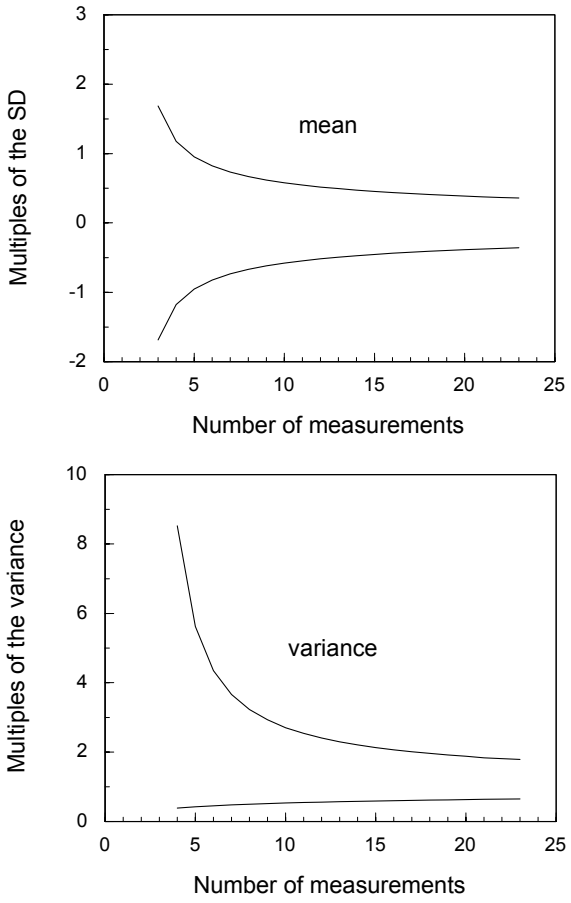

Figure 5.5 The 5th and 95th percentile confidence limits for estimates of the mean and variance as a function of the number of measurements used to calculate the estimates. The limits for the estimate of the mean are expressed in terms of the standard deviation with the estimate located at the origin. The limits for the estimate of the variance are expressed in terms of the variance with the estimate located at 1.

Monitoring rules

Because monitoring studies are typically performed on a more or less regular schedule, there are usually a number of opportunities to evaluate study results following a change in physiologic status. This means that the ability to detect a change in status will be improved because the chances that at least one of the monitoring results will indicate a significant change in marker concentration increases with the number of measurements. However, as the sensitivity to detect a change in concentration increases, the specificity declines. For instance, as presented, the preceding formula for identifying a significant change in marker concentration has a specificity of 0.95. Were this formula to be used in serial monitoring, the specificity would decline which each study repetition,

$$specificity\ of\ n\ measurements = 0.95^{n} \quad .$$

After two determinations, the specificity would be 0.90 and after five determinations it would be 0.77. Clearly the repetitive use of this formula is not acceptable as a monitoring rule.

A number of candidate clinical monitoring rules can be found among the quality control rules used in the clinical laboratory. The 1_{3s} quality control rule, for instance, which stipulates that a monitoring study result must be greater than 3 standard deviations different from the preceding average value to be significant, gives a single study specificity of 0.998

and still has a specificity greater that 0.95 after 25 repetitions of the study. The 2_{2s} rule, which requires two consecutive study results to differ by more than 2 standard deviations from the preceding average value, and the 4_{1s} rule, which requires four consecutive study results to differ from the preceding average value by more than 1 standard deviation, have specificities comparable to the 1_{3s} rule. Although these monitoring rules have similar specificities, they differ somewhat in terms of sensitivity with the 4_{1s} rule showing the best performance and the 2_{2s} rule the worst (Parvin 1991).

Other statistical approaches that have been explored as a basis for the design of clinical monitoring rules include time series modeling using ARIMA (Crabtree *et al.* 1990) and the CUSUM test (Piccoli *et al.* 1987).

MONITORING DISEASE ACTIVITY

Disease activity is monitored primarily with the intention of detecting an increase in activity so that therapy may be instituted or intensified as early as possible and the clinical consequences of the worsening of the disease minimized. Laboratory studies that may be used as markers of disease activity include direct measures of disease activity, such as plasma tumor markers in certain cancers, measures of the physiologic function of organs involved by the disease, such as pO_2 and pCO_2 in pulmonary disorders, and measures of inflammatory or immune response to the disease, such as specific antibody titers in certain infectious diseases. Monitoring the impairment of an organ's physiologic function is a particularly good way to monitor disease activity, when possible, because it simultaneously quantifies the clinical impact of a change in activity.

The advantages of monitoring disease activity using direct markers of disease activity or measures of inflammatory or immune response to the disease are two-fold. First, for many diseases, there is no physiologic function marker that correlates with disease activity. Second, changes in these markers may precede the clinical effects of the change in activity. Thus, these markers can in some cases provide a lead time for a therapeutic response to the change. The major disadvantage of the use of these markers is that the relationship between disease activity and the magnitude of the marker is sometimes unpredictably nonlinear and often varies over time. The relationship can even break down altogether as the disease enters a different pathobiologic stage. For example, a tumor metastasis may not elaborate the marker substance produced by the primary tumor so metastatic progression of the tumor may not be associated with any change in the plasma concentration of the tumor marker.

Because monitoring of disease activity is a serial process with multiple specimens obtained over time, a monitoring rule must be employed to determine when a significant change in marker concentration has occurred. The rules to consider are exactly the same as those for the monitoring of physiologic status. The application of the 1_{3s}, 2_{2s}, and 4_{1s} monitoring rules is discussed as an example.

The results of monthly monitoring of plasma carcinoembryonic antigen (CEA) concentrations in a patient who had primary surgery for breast cancer are shown in Figure 5.6 (the data are taken from Winkel *et al.* 1982). CEA concentrations were monitored because some of the patients who subsequently go on to experience recurrence of their cancer show a significant elevation in CEA concentrations before disease progression is evident clinically. This patient had a clinically overt recurrence of her tumor soon after the last monitoring specimen was obtained. As is easily appreciated from the graphical presentation of the data, the CEA concentrations appear to be fairly constant over the first 13 months, so those results can be used to calculate avg_{indiv} and var_{indiv}; the values are 1.3 μg/L and 0.032 $\mu g^2/L^2$, respectively The critical value for the 1_{3s} monitoring rule is 1.8 μg/L. The CEA concentration at month 14 is 3.5 μg/L, so the concentration increase evident in the result can be considered significant even though the result is far below the population-based critical value of 7.4 μg/L. The population-based critical value was calculated using postoperative CEA concentrations seen in patients who, after long-term follow-up, didn't experience a tumor recurrence. The subsequent results are all also greater than 1.8 μg/L so there is a persistent significant elevation in CEA concentration. The critical value for the 2_{2s} rule is 1.6 μg/L which is exceeded by the consecutive results at months 14 and 15 as well as and all subsequent consecutive result pairs. The 4_{1s} rule's critical value is 1.5 μg/L which is exceeded by the consecutive results at months 14 through 17. Thus, all three monitoring rules demonstrate a significant increase in the CEA concentration. Any of the rules could have been used to detect the CEA elevation in this patient but

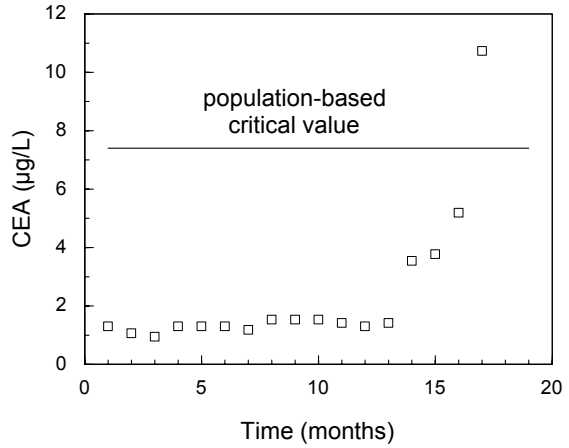

Figure 5.6 Postoperative plasma CEA concentrations in a patient with breast cancer. Data from Winkel *et al.* (1982).

the earliest demonstration of the increase in CEA concentration was achieved by the 1_{3s} rule. This rule gave a significant result 4 months prior to the clinical appearance of the tumor recurrence and 3 months prior to obtaining a significant result based on the population-based critical value. Thus, for this patient, the 1_{3s} rule performed very well and would have allowed for the earliest treatment of the recurrence.

MONITORING THERAPEUTIC RESPONSE

The purpose of monitoring therapeutic response is to determine if the expected benefit has been obtained from a therapeutic maneuver and to gauge the magnitude or risk of any unwanted effects from the maneuver. Clinical observations may provide all the information needed to assess the efficacy and toxicity of medical therapy but, often, laboratory studies are needed to supplement the clinical observations. Indeed, sometimes there are no overt clinical findings that correlate in a timely fashion with the effects of therapy, in which case laboratory findings are the only useful measure of therapeutic response.

Medical intervention is instituted with the intention of curing a disorder or decreasing the intensity or extent of the disorder. Therefore, markers of the level of activity of a disorder, if available, are particularly suitable as tools for therapeutic monitoring. Indirect measures of disease activity, such as organ functional state or metabolic status, can also be highly reliable indices when monitoring therapy. An example is monitoring the reticulocyte count to evaluate the effect of treatment of iron deficiency anemia with iron. The evidence of successful therapy is apparent within days when this measure of marrow generative function is used.

Sometimes neither direct nor indirect laboratory markers of disease activity are available. In such a circumstance, it may be possible to use a measure of therapeutic intensity as a predictor of therapeutic response. This situation arises not infrequently in drug therapy, especially with drugs that are used to control episodic clinical conditions such as epilepsy. This kind of monitoring, called therapeutic drug monitoring, is discussed in a later chapter.

Timing of monitoring studies

Therapeutic monitoring using direct or indirect measures of disease activity should be performed at those times following the institution of therapy that most reliably indicate the response of the marker to the therapy. For the example of monitoring iron therapy in iron deficiency anemia, if one uses the reticulocyte count, the maximal response is seen days after beginning therapy so this is the best time to perform the study. If one uses the blood hemoglobin concentration to monitor iron therapy, a 3 mmol/L (2 g/dl) increment requires approximately 3 weeks, so monitoring with this criterion calls for a study at 3 weeks. If the restoration of a normal hemoglobin concentration is the monitoring criterion, a hemoglobin determination should be performed at 6 weeks. Individually none of these monitoring schemes is fully satisfactory. If one looks only for an initial reticulocyte response to iron therapy, later evidence of coexistent vitamin deficiency or other complicating factors will be missed entirely. If one only looks for the final restoration of a normal hemoglobin concentration, one will miss early evidence of an incorrect diagnosis of iron deficiency. The optimal monitoring strategy here, and generally, consists of a combination of monitoring studies; rapidly responsive markers measured early on to assess the immediate impact of therapy and definitive markers measured after the new physiologic steady-state is achieved to determine if that steady-state is the one expected in response to therapy. If therapy is meant to maintain an altered physiologic state for an extended time, periodic monitoring of the state is also desirable. The timing and frequency of periodic monitoring depends upon many considerations including the therapeutic schedule, the time course of marker

response to therapy, the clinical importance of alterations in the physiologic state, and the turn-around time and expense of the monitoring studies.

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