FOR DEBATE

Onychomycosis: Making Sense of the Assessment of Anti-fungal Drugs

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After years of therapeutic triviality, we now have drugs with a major effect on onychomycosis, and, paradoxically, their very success will lead to the development of more such drugs. This note is concerned with improving their assessment by rationalising the way in which efficacy is measured. The need for this will be apparent to anyone who has had to review studies of antifungal drugs for onychomycosis, especially those done for drug registration. To the more fortunate others, who can use these drugs without having to suffer the tedious analyses of “evidence”, it is necessary only to say that the methodology for the assessment of response is a muddle, illogical in almost all its aspects. I will therefore consider all the procedures involved, examine their weaknesses and suggest some simple improvements. Do not confuse my purpose: this is not a review, nor an “overview”, its duplicitous offspring; while I base my case on an analysis (and increasingly despairing reading) of the literature, I do not intend to anatomise it.

(accepted July 23, 1997.)


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MYCOLOGICAL ASSESSMENT

Reproducibility

The beginning begins with mycological assessment. The usual practice is to study patients with a clinical diagnosis of onychomycosis in whom fungus is subsequently detected by culture and microscopy. Unfortunately the complementary need to establish the reliability of fungal detection is ignored. While, apart from recruitment, this does not affect entry to the trial, it is vital for the assessment of response to know how often loss of fungus on culture and microscopy is an artefact, rather than an effect of treatment. Yet this essential mycological probity is easily established by taking the clinical diagnosis as starting-point and repeating culture and microscopy at short intervals; this would also give a measure of reliability of clinical diagnosis (the frequency of isolation of fungus from clinically normal nails – a desirable figure – will be too low to affect the outcome). Figures based on nail samples sent for routine mycological investigation (1), are unacceptable, as clinicians often send samples for negative confirmation. I can propose a few simpler approaches; one derives from the number of patients with a clinical diagnosis in whom a mycological confirmation cannot subsequently be made, and who are therefore excluded from the trial study. That figure, which is usually wasted and left unpublished, in fact gives a composite measure of the reproducibility of mycology and clinical diagnosis. I found a study which gave the number pretreatment exclusions because of negative mycology; they amounted, surprisingly, to only 5.5% of the total (2). Because spontaneous regression of fungal nail disease is rare, and placebos do not have a direct effect on disease – despite the belief of some mystic medics – another measure of mycological reproducibility is the rate of apparent negativity in the “placebo control” group. This measure will obviously be unacceptable for topical agents whose vehicle itself has a therapeutic effect. Since estimates of reproducibility are as essential to the assessment of mycological as any other cure, it is remarkable that clinical trials are accepted for drug regulatory purposes without this basic piece of information. Here, as elsewhere in this sorry story, there seems to be more concern with the breathlessly difficult mountains of statistical, kinetic, and toxicological travail than the easy flat-lands of therapeutic response.

Dissociation of fungal microscopy and cultural viability by drug therapy

The limitations of fungal microscopy. As with diagnostic and mycological reproducibility, the defects of current practice are less important for disease definition on entry to a study than the subsequent definition of cure. Mycological efficacy is generally measured as time to mycological cure, together with cure rate at a particular, and usually arbitrarily selected, time. It is beyond my understanding why it is not appreciated that, whereas identification by both culture and microscopy can be used for entry to a study, response knows no such twinned necessity. Surely it is obvious that whereas fungal inactivation by drugs renders a culture negative (“cide” and “static”, the twin morticians so beloved by industry, are, in this respect, irrelevant), microscopy does not distinguish living from dead filaments which will continue to be shed; they are trapped in the nail and associated subungal keratin and have no other place to go. Dead filaments, like dead warriors, are the last to leave the therapeutic battlefield, and belief that the battle is over only when the corpses are removed is extraordinary; the current practice of accepting a negative culture only when accompanied by negative microscopy is a monumental, if not sacrilegious, absurdity. For those unmoved by, or suspicious of, too obvious a logic, there is any amount of evidence to support my conclusion: all studies that catalogue findings during treatment show the clear dissociation between loss of cultural viability and the continued finding of microscopically defined filaments (eg. 3–6.)

The limitations of cultural viability. But while it is clear we can no longer rely on simple microscopy as a measure of fungal death, how reliable is culture after antifungal drugs? Could the loss of cultural ability be an artefact, drug collected during sampling inhibiting fungal growth by contamination? Certainly, the
development of reliable new vital staining techniques (these, and histochemically stainable metabolites, would make god PhD project for a young mycologist at the hyphic growing edge) would provide useful evidence of the biological state of the filaments and, in particular, the zone of transition from living to dead. However, the possibility that loss of culturability is an artefact is improbable. It would be necessary to suppose that drug was deposited independently from the fungus, yet close enough to be adventitiously collected with it during mycological sampling. This is anatomically unlikely; moreover, diffusional equilibrium in the nails occurs in days, which also excludes a differential sequestration. Finally, and most importantly, the time course of cultural inhibition is not that of a contamination artefact, which would be apparent as soon as drug was applied or deposited after systemic administration. In fact, my theoretical conclusion is substantiated, inadvertently, by one study (7) in which 28% itraconazole was applied without removal of the drug before nail sampling: loss of culturability was immediate and contamination is presumed. By contrast, the time course of cultural inhibition after systemic drugs (as could also be predicted after topical drugs, when adequately removed before sampling) is slow in onset, increasing gradually to a maximum at about 6 months (e.g. 5, 6). This is several months after therapeutic, or peak levels of drug are reached (e.g. 8, 9). Thus, with the single rule-proving exception, loss of culturability after antifungal drugs cannot be explained by contamination artefact, nor by the kinetics of drug delivery.

It is fortunate that a contamination artefact can so easily be excluded as an explanation of the loss of cultural viability long before loss of filaments as detected by microscopy; otherwise culture could not be used as a method of assessment. Indeed all previous studies, including those used in the registration of all previous drugs for onychomycosis, would be invalid! Once it is appreciated that the contamination argument would kill all previous studies, as well as the poor fungi, opportunism will side with logic, and the argument will disappear. But before it goes, I must note a serious component that remains unresolved: what impresses me, from a survey of all studies which give adequate detail, is that the frequency of cure is far less than might be expected from the observed loss of culturability. Perhaps the most important of the several possible explanations is that loss of cultural viability is relative, and not the absolute measure of fungal death that we now take it to be. What, for example, is the minimal quantity of active fungus required to replicate in culture? When does growth stop after serial dilution of samples of known fungal density? We need quantitative definitions of viability in culture, as well as correlations with vital staining (or metabolic markers of vitality) to establish the number of filaments that are living. Without this, our predictions of therapeutic outcome from mycological changes will remain imprecise. But it must be said that none of these concerns about viability justify the absurd reliance on fungal microscopy. Regardless of whether loss of culturability is absolute, or as I suspect, only a relative marker of viability, it nevertheless has to take priority over standard microscopy, which cannot distinguish living from dead filaments, and cannot therefore be used to measure the response of nails to antifungal drugs.

The need for a new mycology. The current practice of assessing antifungal effect by loss of cultural viability only when accompanied by a negative microscopy, which eventually follows the slow outward procession of filaments that were therapeutically castrated months previously, would be merely risible, were it not that this error has been absorbed into drug trials and regulatory practice, leading to irrelevant and costly study procedures. The use of both culture and microscopy (usually wet KOH preparations) is merely historical; it persists, in part, because of muddled mycological thinking. For example, one study (10) gives crude figures for negative culture with positive microscopy, without consideration of possible previous treatment; it also offers the gratuitous opinion that “microscopy is particularly helpful in the case of infected nails which often fail to produce a positive culture due to the fact that the fungus in the accessible, distal part of the nail is no longer viable” – a marriage of the unlikely with the untented. The degree to which these notions apply to fungal disease will, like some of the other notions I have discussed, require a much more sophisticated and quantitative approach by mycologists to both microscopy and culture in disease, and in the totally different situation after antymycotic drugs, which main or kill the organisms, but leave the field littered with their confusing exo-skeletons (I know of no evidence of breakdown of dead filaments in the nail, nor if anyone has looked for it). So, to summarise the mycological simplifications and improvements I propose: we must measure the minimal quantity of active fungus required to replicate in culture; we can enter patients into studies when both culture and microscopy identify the fungus, (although this speaks less of necessity than the paranoia of belt and braces), but if, out of habit, we continue to use both, we must, at least, plot time to negative culture and filament disappearance separately; the former is a measure of mycological viability, albeit relative, while loss of filaments tells not of fungal death, but the time of passing of the cortège; only a simple new way to detect hyphic viability can give microscopy the kiss of life.

CLINICAL ASSESSMENT

Past methods

So far so simple; yet measurement of clinical response is no less absurd than the failure to recognise the irrelevance of microscopically defined fungal ghosts. Some use shamelessly subjective methods; others use objective measurements which are then abused by a perverse subjectivity. Indeed, the vast and arbitrary range of different “methods” is more a tribute to the vigour of subjective imagination than a passion for scientific simplification. As could be expected, therefore, findings differ widely; this, in turn, explains the need for depressingly large numbers of patients and poorly supervised, dull and costly studies. There have, of course, been many serious attempts to quantify response: Zaias & Drachman (11) measured the outward movement of the midpoint of the junction between normal and diseased nail, and Hay et al. (7) developed the same idea, using area instead of length, to calculate a % rate of nail restitution. The difficulty with area (whether calculated as a percentage change with time or as a function of total nail area) is that, unlike length, change is non-linear because the proximal nail is variously concealed by the folds and does not correspond to the nail area beneath. This is not solved by measuring changes in the area of involved nail (instead of unaffected nail), since this area can be altered appreciably, especially if disease is slight, by nail clipping proximal to the lost onychodermal band. However, what all these methods lack is recognition of how the dynamics of drug
cure must dictate how response is measured, in particular the limitation of disease regression by the competing determinants of inward fungal growth and outward nail growth. Indeed, while some studies now include nail growth rate, none seem to know what to do with it and leave the measurement in an embarrassing limbo. In the absence of a correction of response for nail growth rate, measurements remain specific to each particular nail and patient and cannot be compared between different nails or individuals, other than as grouped means.

**A simple, universally applicable new method for measuring nail response**

The otherwise confusing effect of nail growth rate was used in the method I originally developed to show the effect of short, bolus or “pulsed” courses of a single or repeated dose of antifungal drug (3, 4), a therapeutic regimen communicated to Sandoz in the 80s, who chose to ignore it, and to Janssen, who subsequently developed it for itraconazole. Unfortunately, our studies were blinkered by the concern to demonstrate speed of response to minimal doses, rather than to devise a universal measure of its magnitude. The more rigorous definition I now wish to introduce will, I hope, make amends for that past failure. The principle is as follows: in the common distal onychomycosis, disease grows proximally, mostly in the ventral portion of the plate and the nail bed contribution beneath it; if the causal fungi were to die suddenly, the outward movement of disease could be no faster than the rate of growth of nail and associated subungual keratin. A drug that was 100% effective and cure nail immediately, would therefore allow improvement at the same rate as the underlying rate of growth of nail and associated subungual keratin of the ventral nail which moves with it. A totally ineffective drug will allow inward growth of fungus to continue and either keep pace with or overcome outward nail growth. It follows that the measure of therapeutic efficacy is the ratio of the actual (observed) rate of outward movement of disease to the maximal possible rate, which is the underlying rate of outward growth of nail and associated bed; more simply, this is the ratio

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\text{OUTWARD MOVEMENT OF DISEASE} / \text{OUTWARD GROWTH OF NAIL}
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Movement of disease (\( \pm \)) is measured by the shift, from the nail fold, of the junction between diseased and normal nail; outward nail growth is measured by the independent movement of a mark on the nail over the same period of time. This simple ratio of actual to possible regression can be used to express therapeutic response as the % of Maximal Possible Improvement, or more simply, as disease movement/nail growth in mm/mm for length, or mm\(^2\)/mm\(^2\) for area. This differs from all previous expressions in that it provides an absolute measure of response of onychomycosis to drugs; with the sole exception of proximal disease, to be discussed, it is totally independent of degree of involvement of the nail and the rate of nail growth, and can therefore be used for comparing different nails and different individuals. Furthermore, depending on the sensitivity of the measurements, it should be able to detect an effect in a matter of weeks.

The exception is the problem of proximal nail disease. This is doubly distracting, firstly, and simply, because movement of disease concealed by the proximal fold cannot be measured. Secondly, and more importantly, I would expect involvement of the nail root to reduce nail growth rate, since 80% of nail is produced by the root up to the lunula (12) and this generates forward movement (13). Indeed, it seems probable to me that root dysfunction explains the clinical belief that nails severely affected by fungus grow more slowly; confusingly, slow growth could be effect as well as cause. For the onychomycotic parasite, achieving the root creates a Garden of Eden, from which expulsion by nail growth has been arrested. Consequently, even a totally effective drug will not show a response until the area proximal to the lunula – including the invisible part – is restored sufficiently for growth to be a determinant. For this reason, the response of proximal onychomycosis requires a different method of analysis, in which time is allowed for proximal restitution. However, with this single exception of growth concealment and inhibition by proximal disease, the method I propose remains universally applicable.

The methodology of the independent measurement of nail growth and disease regression is simple in principle and requires only the measurement of movement of a groove marked on the nail surface for the former, and the position of the disease edge for the latter. Both measurements can be made directly on the nail or from photographs, but there are several confusing variables. The immediate problem is that the junction between normal and diseased zones is rarely linear, can be overcome to some extent by making measurements only in the midline (11) (or of longest and shortest lengths) and repeating the measurements in exactly the same place. More precisely, a mean length can be determined from computerised analysis of the junction; but then, it would be little more effort to measure area. Although area is a more accurate measurement than length and therefore provides a better measure of the rate of nail growth and disease regression, there are certain problems in its use; for example, growth changes may appear non-linear because of the shape of the proximal nail exposed by the folds. This and other difficulties can be overcome, but a full technical discussion would be out of place in this simple account of principle. However, by scoring the whole of the junction between normal and diseased nail with a marking diamond, both the rate of nail growth (from the proximal fold) and disease movement (from its initial site) can easily be measured from tracings or photographs, either as length or area. Depending on technical facilities, both length and area will no doubt be measured in future studies, the simplicity of length compensating for its lesser accuracy. Whichever measurement is used is irrelevant to the underlying principle of relating disease regression to nail growth, to derive response as % Maximal Possible Improvement, or mm disease improvement/mm nail growth (mm\(^2\)/mm\(^2\) for area). I see no reason why this simple new measurement and definition of effect should not be used in place of the various subjective clinical estimates, as well as the more objective measures, now used in clinical trials of onychomycosis and for submissions to regulatory authorities.

Of course, no measurement of response, however objective, can displace clinical observation of disease end-point; confirmation of the time to complete disappearance of disease in all its aspects, including subungual keratosis and onycholysis, remains essential. This, coupled with absence of mycological growth and filament disappearance – remembering that dead filament disappearance lags behind unculturability – will distinguish between disease ablation and subsequent healing. In short, the time-consuming, inaccurate and irrelevant clinical
assessments of nail involvement, which are now done with the tedious regularity of a votive offering, are rendered pointless by the accurate measurement of nail growth and disease movement, and the precise relating of the one to the other as the % Maximal Possible Improvement or mm disease improvement/mm nail growth (mm² for area) together with the time to complete nail restoration. Now that the absurdity of relying on the passage of dead fungal filaments is apparent, and there is reason to suppose that loss of cultural viability may be only relative, the clinical measures I propose become even more important as primary assessment procedures.

Relapse and reinfection

My final point concerns reappearance of disease. And here, as a non- mycologist, I remain puzzled by the lack of definition – both conceptual and factual – of the distinctive phenomena of relapse and reinfection. Does this explain the wide variation in the period of follow-up study found acceptable by regulatory authorities? Or, as with me, is the puzzlement not unreasonable? Once again we must ask those devoted to mycology to provide evidence; how else can sensible guidelines be established for times required to define these two processes? This would not be too onerous; initially, no more than eyeball search and screening by microscopy is necessary.

Envoye

Although this essay has grown in the writing, the conclusions are few; they amount simply to a rational conceptualisation of the obvious, which, as anyone can and many will claim, has been around for years. No doubt these ideas will need discussion and refinement, but I hope they are taken up, because I believe they will reduce much unnecessary clinical trial-mongering, and greatly enhance the value of the results from the fewer, but more critical – and more enjoyable – studies.

ACKNOWLEDGEMENT

Sam Shuster has a Leverhulme Fellowship.

REFERENCES


Acta Derm Venereol (Stockh) 78