LETTERS TO THE EDITOR

Stavudine induced macrocytosis

Recently we noted that patients on stavudine among our cohort of HIV positive patients were tending to have an elevated mean corpuscular volume (MCV). This prompted us to perform a retrospective analysis of the case notes of all our patients on stavudine (n = 21, 19 males, two females). Four patients were excluded from analysis where patient had bone marrow failure of unknown origin and was transfusion dependent; one patient was found to have a low folate level secondary to HIV enteropathy; and two patients known to have very high alcohol intakes. The patient found to be folate deficient had only 4 weeks’ treatment with stavudine during which time his MCV rose by 2.4 × 10^9/l. Both patients with a high alcohol intake showed increasing MCV on stavudine: one of 5.3 × 10^9/l over 8 weeks and the other 3.7 × 10^9/l over 5 months. Both female patients had normal thyroid function tests and the male patients were all clinically euthyroid. Vitamin B12 and folate levels were available for 12 patients who had been sampled when the MCV was noted to be elevated and were normal. All the patients were on prophylaxis for Pneumocystis carinii pneumonia (cotrimoxazole, clindamycin or pentamidine) for at least 6 months before starting stavudine and therapy had not been changed in any patient. Apart from these drugs none of the patients was taking any other drugs which are associated with macrocytosis. Eight patients discontinued zidovudine at the point of starting stavudine; in each case the MCV increased where it might have been expected to fall. One patient who was prescribed stavudine did not develop macrocytosis. He was challenged regarding his compliance and admitted to never having taken the drug.

The results are summarised in the table. There is a progressive increase in MCV with the duration of treatment. As this was a retrospective study, MCV values for certain periods were not available for all the patients and some patients have not yet completed 20 weeks on stavudine.

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To the best of our knowledge, macrocytosis has not been reported in association with stavudine until we notified the Committee on Safety of Medicines. The mechanism of zidovudine induced macrocytosis is unknown. Since both these drugs are thymidine analogues and are known to share metabolic pathways, the mechanisms may be similar.

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Accepted for publication 15 July 1997

Progressive increase in MCV related to duration of treatment with stavudine

<table>
<thead>
<tr>
<th>Time</th>
<th>No of patients</th>
<th>Median MCV (x 10^9/l)</th>
<th>Mean increase MCV (&gt; 10^9/l)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17</td>
<td>95-60</td>
<td>3-95</td>
<td>1-15, 6-76</td>
</tr>
<tr>
<td>4-8 weeks</td>
<td>13</td>
<td>97-10</td>
<td>4-71</td>
<td>0-37, 9-04</td>
</tr>
<tr>
<td>8-12 weeks</td>
<td>9</td>
<td>98-66</td>
<td>4-84</td>
<td>0-89, 15-99</td>
</tr>
<tr>
<td>12-20 weeks</td>
<td>7</td>
<td>105-05</td>
<td>10-47</td>
<td>4-10, 16-84</td>
</tr>
<tr>
<td>&gt;20 weeks</td>
<td>7</td>
<td>112-00</td>
<td>12-44</td>
<td>6-47, 16-82</td>
</tr>
</tbody>
</table>

Biopsy of male genital dermatoses

In 1993 a paper was published in *Genitourinary Medicine* on the experience of genitourinary physicians in the diagnosis of penile dermatoses and the usefulness of penile biopsy.1 Of 71 patients seen over a 1 year period, 60 (85%) underwent a penile biopsy.2 At the time the diagnosis was not made on clinical grounds. A clinical diagnosis was made in nine patients and penile biopsy considered unnecessary. Histological findings were consistent with the initial clinical diagnosis in 33% of the 60 patients undergoing a biopsy and it was concluded that diagnosis based on clinical appearance alone is inadequate. We would like to report our experience with the use of penile biopsy within the setting of a special penile dermatoses clinic in the dermatology department in which the patients were assessed by a dermatologist with an interest in dermatoses of the male genitalia.

A specific monthly clinic was set up in the dermatology department in 1993 for the diagnosis and management of men with penile dermatoses. Patients are referred to the clinic by their genitourinary physicians including those in the genitourinary clinic, the general dermatology clinic, dermatologists from other hospitals, and general practitioners. The clinic is run by a dermatologist with an interest in disease of the genitalia (GB) and attended by a genitourinary physician (DH) and, more recently, a urologist (MD).

In all, 286 patients have been assessed over a 4 year period. Patients ranged in age between 18 and 93 years with the exception of two patients who were 4 and 12 years old. The majority of patients (n = 223) ranged in age between 18 and 50 years. The commonest presenting conditions were psoriasis (n = 68), penile infections (n = 47), seborrhoic dermatitis (n = 26), lichen sclerosus (n = 36), lichen planus (n = 28), Zoon’s balanitis (n = 23), and eczema (n = 21). Less common diagnoses were vitiligo (n = 5), irritant contact dermatitis (n = 9), lichen simplex (n = 6), allergic contact dermatitis (n = 3), Bowen’s disease (n = 3), Bowenoid papulosis (n = 3), squamous cell carcinoma (n = 1) and balanoposthitis (n = 2), idio-pathic penile balanitis (n = 2), and circinate balanitis (n = 1).

In most cases (n = 218, 77%) a clinical diagnosis was reached without the need for a penile biopsy. Of all patients (n = 218) and those 20% (n = 436) who were biopsied: 19/36 (53%) patients with lichen sclerosus were biopsied of whom six (32%) had a biopsy performed to elucidate the diagnosis as a firm diagnosis could not be made on clinical grounds; 13 patients with lichen sclerosus (68%) had a biopsy performed to confirm the clinical diagnosis and assist clinical management; 17/23 (74%) of patients with Zoon’s balanitis were biopsied, in cases to confirm the clinical diagnosis; two patients with a clinical diagnosis of Zoon’s balanitis and lichen sclerosus had dual pathology confirmed histologically; 10/28 (36%) patients with lichen planus were biopsied, of these four (40%) were biopsied to elucidate the diagnosis because of clinical uncertainty, while six were biopsied to confirm the clinical diagnosis; 5/21 (24%) patients with eczema, 4/30 (13%) patients with viral warts, 2/68 (3%) of patients with psoriasis were biopsied, in each case to confirm the clinical diagnosis. All biopsies were performed at clinics, the need for biopsy was made in all cases of seborrhoic dermatitis, lichen simplex, allergic contact dermatitis, idiopathic oedema, vitiligo, and in the case of circinate balanitis.

There was a very high concordance between clinical diagnosis and histological diagnosis and in only two cases did the findings result in a change in the diagnosis. In both of these a clinical diagnosis of lichen sclerosus was made while in one feature of lichen planus were present historically and in the other the histological findings were non-specific.

We have experience most patients with inflammatory penile disease such as psoriasis, eczema, lichen simplex, contact dermatitis, and lichen planus have cutaneous signs at extragenital sites and a full examination enables a firm diagnosis which obviates the need for biopsy. The presence of extragenital cutaneous inflammatory skin disease helps to corroborate the diagnosis.

Most dermatoses of the male genitalia are amenable to clinical diagnosis reached on classic dermatological grounds of full history taking and complete physical examination. Penile biopsies do not need to be performed routinely although they may be useful in confirming the clinical diagnosis. Secondly, a histological diagnosis may be valuable in advancing patient management—for example, increasing the authority with which surgery is advocated in diseases such as lichen sclerosus and Zoon’s balanitis where circumcision may be necessary.

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Accepted for publication 15 July 1997

Characterisation of high level tetracycline resistant *Neisseria gonorrhoeae* isolates

Three of 1039 clinical isolates from consecutive patients with urethritis, who attended urological or STD clinics in Tokyo and Kanagawa area between 1985 and 1995, were determined as tetracycline resistant *Neisseria gonorrhoeae* (TRNG). These strains had minimum inhibitory concentrations (MICs) of >16 mg/l and gave a zone of inhibition of < 30 mm from the edge of the 30 µg tetracycline disk.
cine disc to the edge of confluent growth (table). In this study, we further characterised the tetM genes of these TRNG strains.

Ison et al. previously reported the primer pair (A: 5'-GGGTACCAAGC-AACATGGCTC-3' and B: 5'-TTCCTTGTACGTTACCTCG-3') for detection of tetM in N. gonorrhoea. These sequences were derived from that of the Ureaplasma species. More recently, the nucleotide sequences of the tetM genes of American and Dutch type plasmids have been determined and suggested that the tetM determinant found in the American type plasmid has a different origin from that in the Dutch type.2 Because the base sequence of the tetM gene from Dutch type plasmid which corresponds to the primer B is different from that of American type plasmid,1 primer B (5'-TTCCTTGTACGTTACCTCG-3') was used instead of the primer B to detect Dutch type tetM. The cells grown on a Kellogg's agar medium were lysed in 100 µl of distilled water for 10 minutes at 94°C. The resulting solution was added to a polymerase chain reaction (PCR) mixture. The PCR mixture contained 0.2 mM (each) deoxynucleoside triphosphate, 50 pmol of each oligonucleotide primer, TaKaRa Taq DNA polymerase (TaKaRa Shuzo, Kyoto, Japan), and buffers provided by the manufacturer in a total volume of 50 µl. The mixture was overlaid with 50 µl of mineral oil and heated in a DNA thermal cycler PF-2000 (TaKaRa) for 25 cycles consisting of 45 seconds at 94°C, 60 seconds at 58°C, and 60 seconds at 72°C.

PCR amplification using the primer pair of A and B gave a product of the predicted size, 785 bp. The PCR products from strains 60061 and 6010 from 3120 and 6010. On the other hand, the primer pair of A and B amplified a 765 bp fragment from strains 5120 and 6010 but not from 60061. The restriction digests using MspI of the PCR products of 60061 gave the predicted three fragments of 370, 260, and 140 bp. MspI digests of the PCR products of 5120 and 6010 generated three fragments of 540, 140, and 90 bp. The amplified products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Detection Kit (Perkin-Elmer Corp CT, USA) and the ABI 310 Genetic Analyzer (Perkin-Elmer Corp), and that from 60061, 6010 and 5120 were identical to corresponding sequence of the tetM gene from American and Dutch type plasmids, respectively.

It is of interest that the isolation rate of TRNG was quite low and both American and Dutch type tetM genes were found in Tokyo and Kanagawa, Japan during the study. The strain with a strain pattern isolated in 1985 was infected in Japan. A strain 5120 was imported from Thailand (table). These facts imply that TRNG already existed in 1985 in Japan and has been transported from other countries, but not spread in Tokyo and Kanagawa area. Ison et al. found two types of HpaII (MspI) digestion pattern of the PCR products from tetM in TRNG strains. In this study we clearly distinguished the American type tetM gene from the Dutch type one using the PCR with the sets of the primer pairs. Further investigations will be needed to elucidate the prevalence of each type of tetM gene in N. gonorrhoea infections.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolation date</th>
<th>Source</th>
<th>MIC (mg/l)</th>
<th>Disc diffusion test* (mm)</th>
<th>tetM type</th>
</tr>
</thead>
<tbody>
<tr>
<td>60061</td>
<td>1985</td>
<td>Yokohama</td>
<td>16</td>
<td>15-0</td>
<td>American</td>
</tr>
<tr>
<td>5120</td>
<td>1993</td>
<td>Holland</td>
<td>10-0</td>
<td>32</td>
<td>Dutch</td>
</tr>
<tr>
<td>6010</td>
<td>1994</td>
<td>—</td>
<td>12-0</td>
<td>32</td>
<td>Dutch</td>
</tr>
</tbody>
</table>

* A zone of inhibition from the edge of the tetracycline disc to the edge of confluent growth. This conclusion was based on the results of two large general practice prevalence studies which were not quoted by Simms et al.6 Six studies were identified from a review of recently published randomised control trials published in the British journal. Further trials are needed in the United Kingdom. The present study is in agreement with that of Simms et al.1

MATTERS ARISING

The discrepancy between my findings and those of Simms et al. supports the argument made by the Evdence1 and British family health care Working Group that all reviews of the medical literature should make explicit how the cited literature was obtained, sifted, and appraised.1 Failure to do so is likely to lead to important papers being missed.


Replay

Dr Stokes raises a number of important points that correctly points out the method of assessing the literature was not fully explained. The paper aimed to provide a concise, comprehensive, and timely review of what is a rapidly expanding field. A literature search was carried out using Medline and by treading the literature. The search excluded to England and Wales (one review cited by Dr Stokes was concerned with the United Kingdom as a whole). The literature search excluded to England and Wales (one review cited by Dr Stokes was concerned with the United Kingdom as a whole).

To ensure comparability between studies each paper was assessed in terms of the population studied, the presentation of results, and the testing strategy used. Studies were only included where the raw data were presented in a way that allowed the diagnostic test to be calculated. Diagnostic tests have undergone a rapid evolution over recent years and a wide variety of tests and testing strategies have been used. It is difficult, if not impossible, to make comparisons between all articles. We decided to include only those studies that used a strategy based on screening and confirmatory tests.

Studies undertaken by physicians, micro-

Epidemiology of genital Chlamydia trachomatis

Simms et al.1 in their review of the epidemiology of genital Chlamydia trachomatis in England and Wales state that "ad hoc prevalence and case finding studies carried out over the past 20 years were critically assessed in terms of study design and testing methodologies. The authors, however, do not define what is meant by "ad hoc" and do not make explicit how the cited literature was obtained, sifted, and appraised. As a consequence they fail to identify all relevant published prevalence studies."

I recently reviewed the literature relating to the prevalence of G. trachomatis infection in women attending British general practices (which updated an earlier review of the literature2) and summarised the findings in a recent paper.3 As I wished to ensure that all relevant studies were included I used an explicit search strategy and stated the reasons for inclusion or exclusion of the identified studies. This led to 15 relevant prevalence studies being identified and appraised. Simms et al., in contrast, identified only six of these studies.

As far as general practice was concerned nine studies which met defined criteria were included in the review. It was concluded that the best current estimate of the prevalence of genital chlamydia in women attending general practice is 3% to 4% (range of 1% to 10%).4 Further studies have been done in family planning clinics in the United Kingdom and Wales. These studies estimated prevalence rates from 3% to 10%.5 The epidemiological evidence suggests that genital chlamydia infection in women is currently underestimated in both general practice and family planning clinics, although there is a common trend over recent years. Further studies are needed to confirm this finding.
Characterisation of high level tetracycline resistant Neisseria gonorrhoeae isolates.

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Genitourin Med 1997 73: 421-422
doi: 10.1136/sti.73.5.421-b