RHEUMATOLOGY: PROBLEM, PROMISE AND PITFALLS*

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This paper is intended to discuss a number of topics in which there have been significant advances in knowledge in relation to the treatment of patients suffering from inflammatory joint diseases.

PROBLEM: NSAIDS AND THE GUT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are efficacious and often a 'godsend' to rheumatology patients, but their safety is a major concern to the patients and their doctors. NSAID-related peptic ulcer complications such as haemorrhage and perforation are serious problems; predisposing factors include increasing age, previous peptic ulcer disease and higher drug dosage. In the UK 2,431 gastrointestinal (GI) bleeds and 280 deaths were associated with NSAID use over one year, and these figures are likely to be conservative. Toxicity tables for various NSAIDs have been constructed; Langman *et al.*[1] assessed the risk of being admitted with an acute upper GI haemorrhage in terms of odds-ratios for different NSAIDs and found a ten-fold variation in risk from the highest, azapropazone and ketoprofen (23.7), to the lowest, ibuprofen (2.0). Complications could be substantially reduced by changing the NSAID therapy of those requiring such medication to a drug with a favourable odds-ratio for GI haemorrhage risk. Serial endoscopy in healthy volunteers or patients are often used to assess the GI toxicity of NSAIDs, and the benefit to be derived from concomitant administration of gastroprotective agents, but it is controversial as to whether the acute ulcers visualised at endoscopy are clinically relevant, especially in relation to more sever gastrointestinal complications.

Having commenced a patient on a NSAID with a favourable odds-ratio for GI haemorrhage risk, is there evidence to support the requirement for using gastroprotective agents to further reduce this risk? The 1995 MUCOSA study[2] from the US, involving over 8,000 patients with rheumatoid arthritis (RA) on NSAID therapy, assessed the gastroprotective effect of misoprostol, compared with placebo over six months. A significant reduction was observed in the incidence of perforation and gastric-outlet obstruction, but not in bleeding (which is the most clinically important problem associated with NSAID use). How applicable the results of this study are to the UK population is debatable, as 40% of the patients in it were on concomitant oral steroid therapy, and there were many exclusion criteria in the study, e.g. women of childbearing age. In addition, 40% of the study subjects on misoprostol withdrew because of side-effects. Thus misoprostol is not the solution to NSAID-related GI toxicity.

What then is the answer to the GI problems associated with NSAIDs? Cyclooxygenase-2 (COX-2) selectivity of NSAIDs has been sought as a potential solution, and hopes have been raised by the recent launch of COX-2 specific agents. These drugs are the third generation of NSAIDs and they have become available 100 years after the first generation, i.e. aspirin. Sir John Vane showed that inhibition of prostaglandin synthesis was central to both the actions and side-effects of aspirin. The pharmacological target of NSAIDs is cyclooxygenase which catalyses the first committed step in arachidonic acid metabolism to prostaglandins. Two isoforms of the enzyme are known: COX-1 is constitutively produced and serves housekeeping functions in the kidney, cardiovascular system, GI tract and platelets, whereas COX-2 is induced at sites of inflammation and contributes to the process. The COX enzyme is shaped like a double shell and occurs in membranes around a channel into which arachidonic acid passes. The channel in the COX-2 enzyme is wider and contains a side-pocket. NSAIDs bind reversibly to these sites by hydrogen bonding and block the channel. However, NSAIDs with selectivity for COX-2 are bulky molecules which are too large to enter the COX-1 channel but are able to bind irreversibly to the side pocket of the COX-2 enzyme.[3] A number of ways are available to assess COX-2 selectivity and these give different quantitative results; one of the most useful is the human whole blood assay.

Meloxicam has increased COX-2 selectivity and clinical trials have shown fewer GI adverse events compared with diclofenac, although the selectivity does diminish at higher doses.[4] Drugs which are specific for COX-2 have been sought and the two leading compounds are rofecoxib and celecoxib. The use of very high concentrations (up to 80 times the starting dose for osteoarthritis) of rofecoxib[5] showed no effect on COX-1 in healthy volunteers but substantial inhibition of COX-2, and celecoxib also seems to have no effect on COX-1 at therapeutic concentrations. In addition prostaglandin production in gastric biopsies was unaffected by rofecoxib. Rofecoxib has been found to have a similar efficacy to ibuprofen in treating post-dental surgery pain.[5] The efficacy of rofecoxib and celocoxib in osteoarthritic pain appears to be similar to existing NSAID,[6,7] and both drugs are licenced for this usage in the US. In a phase III trial of patients with RA, celecoxib showed similar efficacy to naproxen,[8] and celecoxib is now also licenced for RA in the US.

If efficacy is similar to existing NSAIDs what then of GI safety? A seven day endoscopy study in volunteers showed a similar incidence of gastric erosions after administration of ten times the clinical recommended dose of rofecoxib compared with placebo. A separate study showed no increase in faecal occult blood loss with rofecoxib over four weeks. Placebo-controlled endoscopy studies over three or six months of both rofecoxib and celecoxib have shown a significant reduction in gastroduodenal damage compared with non-selective NSAIDs.[9,10]

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The important clinical issue is not so much the occurrence of erosions and ulcers seen at endoscopy but the much rarer and more serious ulcer complications. The incidence of upper GI perforations, symptomatic ulcers and upper GI bleeds (PUBs) has been compared in patients taking rofecoxib (N=3,357) with those taking NSAID (N=1,564) in a pooled analysis of eight osteoarthritis trials. The 55 PUB events were analysed by an external, blinded case-review committee using stringent, pre-specified case definitions to determine whether events were confirmed. The cumulative incidence curve of confirmed PUBs over 12 months was significantly lower with rofecoxib compared with NSAID (odds ratio 0.45 95% CI 0.25, 0.81).[11]

Although these results are promising, whether COX-2 inhibitors will prove to be the answer to the problem of GI safety with NSAIDs remains to be shown with longer-term clinical experience. In addition the issue of non-GI side-effects (renal, hypertension, fluid retention) with these new agents remains of concern and requires further investigation.

PROMISE: NOVEL THERAPIES

Leflunomide – a novel therapy for rheumatoid arthritis

In RA, the inflammatory infiltrate in pannus produced by activated T cells is important in both the initiation of disease and in established disease.[12] Cytokines (γIF/IL2, IL1, IL6, TNFα) produced by activated T cells induce macrophage-like and fibroblast-like cells to produce the destructive metalloproteinase enzyme, gelatinase, responsible for cartilage destruction.

Leflunomide (LFU) is a disease modifying anti-rheumatic drug (DMARD) with a novel mechanism of action in rheumatoid arthritis; it has been licensed in North America and received the Committee for Proprietary Medicinal Products approval in London in May 1999 but is awaiting final approval in Europe. Leflunomide is an isoxazol derivative and its mode of action is to inhibit a pivotal step in *de novo* pyrimidine synthesis (and hence nucleic acids) by inhibiting the enzyme dihydroorotate dehydrogenase (DHODH). Activated lymphocytes are heavily dependant on pyrimidines and have a seven-to eight-fold increase in demand for pyrimidines by the *de novo* pathway compared to resting lymphocytes which mainly use the 'salvage pathway' in which DHODH is not involved. The imbalance between nucleotides in activated cells leads to cell cycle arrest via activation of p53, but not to cell death. *In vivo* studies have shown that the bone marrow, skin and gut are not affected by LFU which is different to most other DMARDs, especially the cytotoxics. In a recent series ~31% of DMARDs were discontinued due to side-effects. Leflunomide also inhibits nuclear factor κ B (NFκB) activation in TNF-stimulated cells, thus resulting in reduced expression of IL2 and IL1β (reduced pro-inflammatory events), favours tissue inhibition of metalloproteinases and inhibits metalloproteinase production (enhanced / upregulated anti-inflammatory events).[13]

The six-month data from a multicentre randomised double-blind placebo-controlled phase III clinical trial comparing sulphasalazine (SZP) (0.5g daily titrated to 2g daily at week four) with LFU (100mg daily for days one to three then 20mg daily) has been published.[14] Forty-one per cent of patients had RA duration of ≤ 2 years, i.e. early disease, and 40-53% had no previous DMARD. American College of Rheumatology (ACR) response rates to both SZP and LFU were similar and significantly better than placebo. For LFU the ACR20 was 55% versus 56% for SZP and 29% for placebo and the ACR50 values were 33%, 30% and 14% respectively. An improvement in health assessment questionnaire and reduction in progression assessed radiologically were also seen. When the study was extended to 12 months the improvement was continued. Withdrawals at 6 and 12 months from the LFU arm were 8% and 5% due to lack of efficacy, and 14% and 3% due to side-effects. SZP had a higher withdrawal due to adverse events. In early disease (≤ 2 years duration), ACR20 response was 58% for LFU versus 41% for SZP, and for a disease duration > 2 years the values were 52% and 60% respectively.

In a 12 month placebo-controlled study comparing LFU with methotrexate (MTX)[15] ACR20 responses of 53% for LFU and 46% for MTX were observed. Combination therapy by the addition of LFU to patients on MTX who have not responded (mean dose 17.2mg weekly) showed a 45% ACR20 response although three patients were removed due to abnormal liver function tests (LFTs), and this may indicate an increased chance of liver toxicity from combined treatment.

The most common side-effect of LFU is diarrhoea, seen in 17%; nausea occurred in 10%, a rash in 10% and alopecia in 8%.[14] In the MTX combination study[15] rarely did the liver function tests reach levels greater than three times normal. No severe side-effects of agranulocytosis (1.5% SZP patients) or pneumonitis (>1% of MTX patients) were seen with LFU.

In summary, LFU is the first pyrimidine synthesis inhibitor which has a unique mode of action among DMARDs. It has a simple, once daily oral dose regimen. It has been shown to improve signs and symptoms of RA and retards radiologically defined progression and improves health-related quality of life parameters. Leflunomide is well tolerated with no serious adverse effects and only a very small percentage of patients require dose reduction due to side-effects.

Stem cell transplantation for rheumatic diseases

New therapeutic strategies have to be developed for the rheumatic diseases because these conditions lead to long-term disability, shorter life-expectancy and high economic cost; the existing treatments are non-curative and may lead to organ damage. Haemopoietic stem cell transplantation is a new therapeutic option for severe auto-immune diseases[16,17] and the aim of such treatment is to reset the immune system and re-introduce tolerance. Allogeneic (from other animals) bone marrow transplantation (BMT) has reversed experimental auto-immune diseases in animal models and similarly syngeneic transplantation (from immunologically and genetically identical animals) can also cure auto-immune disease. The coincidental observation of improvement or cure of auto-immune diseases in patients who had a malignancy treated with bone marrow transplantation further supports the rationale of stem cell transplantation in the rheumatic diseases.[18] Remission of auto-immune diseases for up to 13 years has been observed in patients with BMT for malignancy or drug-induced marrow aplasia.

The process of stem cell transplantation involves patient selection, screening, mobilisation of stem cells, harvesting,

graft manipulation (lymphocyte depletion), conditioning (ablative therapy), re-infusion of stem cells and follow-up. Stem cells may be mobilised from the marrow using granulocyte colony stimulating factor (GCSF) with or without a bolus of cyclophosphamide. Initially there was concern that the bone marrow of RA patients may not behave similarly to that of cancer patients because of prior exposure to multiple toxic drugs and marrow suppression from pro-inflammatory cytokines. However, it has been demonstrated that stem cells can be mobilised from the bone marrow of RA patients in sufficient numbers and that this can be done without initiating a flare-up of RA.[19] Stem cells obtained from peripheral blood are harvested by CD34 antibody positive selection and T cells can be removed by a variety of methods. The patient then undergoes ablative therapy (conditioning) using high-dose cyclophosphamide. Currently there is most variation between BMT protocols in conditioning and some units use total body irradiation, antibodies such as antithymocyte globulin, or busulphan. The stem cells are then re-infused and the patient given supportive care. This procedure is best carried out in units familiar with handling such cases in close collaboration with the haematologists.

A register of patients has been established under the auspices of the European Group for Blood and Marrow Transplantation (EBMT) and the European League against Rheumatism (EULAR). The American register also collects the same core data set. All patients undergoing stem cell transplantation are registered at the time of mobilisation so as to avoid retrospective inclusion of only the positive results. The database will allow monitoring of safety and efficacy, and correlation between the techniques used and outcome. There have been 127 stem cell transplants in Europe, 66 of which were for musculoskeletal diseases including systemic sclerosis (29), juvenile idiopathic arthritis (14), RA (13), and systemic lupus erythematosus (SLE) (10).

The Leeds protocol is a standardised protocol which has been accepted by EBMT. It is considered for patients with severe, active RA, with reversible disability for which no other conventional treatment is available and who have no significant major organ disease. Out of around 100 patients who have been screened, ten have been considered physically and psychologically suitable. Any suitable patient is reviewed by two independent consultant rheumatologists and a consultant haematologist not involved with direct patient care, and informed consent is required. Young people tolerate the procedure best and have most to benefit. The outcomes under investigation include safety, efficacy, immune reconstitution, clinical measures, remission, high resolution ultrasound and arthroscopy. Two patients with RA (a 39-year-old male and a 23-year-old female) have undergone bone marrow transplantation according to the Leeds protocol; one is well at 12 months on a small dose of cyclosporin and the other has shown a greater than 50% improvement at six months.

In summary, autologous bone marrow stem cell transplantation is starting to be used in highly selected patients with severe auto-immune diseases for which there is no other treatment available and a reduction in disease activity can be achieved. It is hoped that data collection by the European database will lead to the development of proper comparative protocols for evaluation by next year.

Monoclonal antibodies to TNF-Alpha

Animal models in the 1950s and 1960s identified a factor released into serum (tumour necrosis factor, TNF) which lead to regression of sarcomas implanted into the skin of mice. In the 1980s, TNFα was cloned and sequenced in the US. TNFα production is upregulated in the pannus of the RA joint. TNF binds to chondrocytes in the synovium, fibroblasts and endothelium, and has a pivotal role via activation of NFkB and mitogen-activated protein kinase pathways to stimulate the series of events leading to inflammation, most importantly the cytokine cascade. Experimental models both *in vitro* and animal models have shown that blockade of TNF by monoclonal antibodies (mAb) leads to remission of disease. This occurs via inhibition of the cytokine cascade which otherwise would activate matrix metalloproteinases leading to connective tissue degradation. Recruitment of bone marrow-derived cells into sites of inflammation and neovascularisation in rheumatoid tissue is also inhibited.

Anti-TNFα blocking agents may be either mAbs or soluble receptors. Infliximab (Remicade) is a humanised chimeric mouse anti-TNFα mAb which neutralises TNF activity in animal studies both *in vitro* and *in vivo*. In the first randomised multicentre placebo-controlled trial of Infliximab monotherapy,[20] 25 patients in either the placebo, low dose (1mg/kg) or high dose (10mg/kg) arms received a single intravenous bolus. A subgroup of patients who received an intermediate dose (3mg/kg) consisted of placebo patients who had relapsed. There were remarkable clinical and biological effects with reduction in tender joint count by 60-70% in four weeks as well as reduction in other parameters such as swollen joints, pain score and patients' own assessment of severity. C-reactive protein normalised within 24-48 hours. There were dose-related response durations (based on Paulus 20 criteria) of three weeks (1mg/kg), six weeks (3mg/kg) and eight weeks (10mg/kg). The treatment was therefore not curative and repeated doses would need to be administered. At that time no other experience of repeated mAb exposure in chronic disease treatment was available for comparison. As the mAb was partly murine it was not known whether repeated doses would be tolerated immunologically. In a multicentre randomised trial of Infiximab as combination therapy,[21] 101 patients who were resistant to MTX were stabilised on a dose of 7.5mg weekly and randomised to either monotherapy with 1mg, 3mg or 10mg mAb +/- MTX 7.5mg or placebo. Low-dose monotherapy led to loss of response but with concomitant MTX administration a sustained response was seen which continued beyond the observation period. This indicated a synergistic interaction between MTX and mAb. One explanation is that the immunogenicity of the chimeric mAb is altered by high-dose therapy or by MTX. The ATTRACT trial is an ongoing combined US and European study of Infliximab among 428 patients with recalcitrant RA resistant to MTX – median dose 15mg for ≤ 6 months. The median duration of disease was 8.4 years, so this is quite different to the trials with LFU discussed above. Patients had also been on a median of two other DMARDs other than MTX and 60% were on oral prednisolone. Patients received either placebo or 3mg/kg or 10mg/kg at 0, 2 and six weeks then four or eight weekly. The first six month data showed a 50-58% ACR20 response in clinical and laboratory parameters for both 3mg/kg and 10mg/kg infusions.

Infliximab is generally well tolerated. A reaction to the infusion occurs in 5% of cases, usually early in treatment, which can be controlled by slowing the infusion and prescribing antihistaminics. No serious reactions leading to discontinuation of the infusion have been reported, although theoretically anaphylaxis could occur. In 8% of cases asymptomatic low titre anti-double stranded DNA antibodies developed with two cases of lupus-like disease. One might expect as TNFα plays a role in host defence mechanisms that treatment would carry an increased risk of infection; however, there was no increase in incidence over controls of serious infections although this is at yet based on early data. Concerns that inhibition of TNF's role in immune surveillance of tumours might lead to an increased risk of malignancy were not supported as no increased incidence in tumours was detected; however, there is a high background incidence of malignancy in the RA population.

Etanercept (Enbrel) is a soluble TNF receptor inhibitor (TNF R:Fc fusion protein) self-administered subcutaneously twice weekly. Trials have shown similar efficacy to Infliximab with ACR20 responses of 59% after six months of monotherapy (11% for placebo)[22] and 71% at 24 weeks for patients who failed MTX (27% for placebo).[23] Other compounds are on trial currently.

In summary, these drugs have proven efficacy and a good safety profile as assessed by symptom control, however their DMARD activity is not known. They are costly: US $12,000 per year for enbrel, US $6,000 per year for Infliximab 3mg/kg eight weekly. They have been proven to be useful for recalcitrant RA patients who have failed other treatments, but their activity in early RA is not known. Licensing status for etanercept was approved by the United States Food and Drug Administration (US FDA) for treatment of RA in November 1998 and is expected in Europe by the end of 1999. Infliximab has been submitted to the US FDA for RA in 1999 and already is licenced for use in Crohn's disease. Efficacy of Infliximab is greater than one year as shown by the ATTRACT data from EULAR. Monitoring is much the same as for other DMARDs. Vigilance for masking of infection is recommended. A few deaths have occurred from infection of the subcutaneous route when it was self-administered. Enhanced supervision is required by centres giving infusions in order to recognise infusion reactions. In 30-40% of patients, no response to anti-TNF treatment is seen. It is postulated that these patients may have a different RA phenotype with another cytokine dominantly involved. The genetics of response to therapy is an exciting field of research for the future. There are no data for delay in radiological disease progression from TNFα blockers as studies have been on patients with eight to ten year median disease duration rather than early disease; however, the one year ATTRACT data may address this issue.

Anti-microbial therapy for rheumatoid arthritis

Occult infection has been proposed as a cause of RA since the 1920s, and doctors have used tetracycline in RA with anecdotal and uncontrolled data supporting its use. In the 1980s researchers started to evaluate minocycline (a tetracycline derivative) because of its calcium-chelating properties. They envisaged that minocycline might be advantageous in diseases such as RA, involving metalloproteinases, as release of these collagenase enzymes is highly cationic-dependent. The gingival fibroblasts of diabetic rats produce large quantities of collagenases and it was found that the collagenase production of fibroblasts from animals administered minocycline in their drinking water was significantly reduced. In animal models of RA, minocycline administered in the drinking water lowered the incidence of arthritis induced by collagen or adjuvant immunisation. Calcium entry is a pre-requisite for full T cell activation to be achieved and minocycline may have an immunosuppressive effect by lowering extracellular calcium and reducing calcium entry into cells.

The hypothesis that minocycline may have an anti-rheumatic effect by altering degradative and immunological pathways led to its evaluation in clinical trials. The MIRA trial[24] compared minocycline (200mg daily) with placebo over 12 months in 219 RA patients at six centres with an average disease duration of nine years. There was a statistically significant reduction in the swollen joint count and erythrocyte sedimentation rate (ESR) with no serious side-effects observed. O'Dell[25] performed a randomised, double-blind placebo-controlled trial in 46 sero-positive patients with RA of less than 12 months duration. After six months, 15 of the 23 patients (65%) treated with minocycline, and 3 of 23 patients (13%) treated with placebo ($p<0.001$) maintained at least a 50% improvement, and five were in remission at one year on minocycline versus one in the placebo group. Trentham[26] reported his open experience with minocycline in 80 RA patients over six years and he found minocycline effective in around one third of patients (with 10% close to remission), ineffective in around one third and not tolerated in one third. Gastrointestinal symptoms and dizziness were the commonest side-effects observed, followed by hyperpigmentation and taste disturbance. Hypersensitivity pneumonitis and drug-induced SLE were very rare.

It is suggested that minocycline is started at 50mg bd and after three weeks escalated to 100mg bd to avoid early side-effects. The main long-term toxicity is hyper-pigmentation (slate-grey discolouration) although this has occurred as early as three months but it does tend to reverse eventually on discontinuation. Minocycline may also have steroid-sparing properties and can be used in combination with MTX to allow the use of a lower dose of MTX.

It is unlikely that minocycline will ever be approved for RA by the US FDA because it is a generic drug and no pharmaceutical company is therefore likely to finance the necessary trials for licensing to be obtained.

In summary, minocycline appears to be a relatively safe, additional therapeutic agent for RA of which physicians are becoming increasingly aware.

PITFALLS: INTERPRETATION OF RESULTS

Positive antinuclear factor

Systemic lupus erythematosus (SLE) has a significant morbidity, mortality and impact on quality of life and well-being. It is a challenge to diagnose and difficult to manage successfully. However, SLE is rare with an incidence of 1.8-7.6 *per* 100,000 *per annum* and prevalence of 6-50 *per* 100,000. Conversely, locomotor disease is the second commonest cause for consultation in general practice in England and with 27,000 consultations *per* 100,000 population, it is the commonest cause for referral by general practitioners to a specialist (1,700 referrals *per* 100,000 consultations). Therefore, detecting cases of SLE among

these numerous referrals is not an easy task, especially in view of the fact that the symptoms of connective tissue disease may be relatively non specific. The ACR criteria for the diagnosis of SLE[27] are difficult to apply clinically in the outpatient setting, for example there are 120 different combinations of 4/11 positive criteria. Also, performance of ACR criteria for SLE against rheumatic disease other than RA, scleroderma and dermatomyositis has not been tested.

Available data suggest that the presence or absence of key clinical features may be the most useful method of discriminating patients with SLE from those without. Among 230 patients presumptively referred for and / or diagnosed with SLE,[28] 10% did not meet the ACR criteria for SLE but were nevertheless clinically diagnosed as having SLE by three independent rheumatologists. In addition, 16% of patients had fibromyalgia-like symptoms and a significantly positive (titre >1:80) antinuclear factor (ANF). Arthralgia (but not arthritis), myalgia, fatigue, depression, insomnia and mucocutaneous features were common among fibromyalgia patients with a positive ANF whereas visceral and haematological abnormalities were rare. Thus these latter features may be more discriminatory for true SLE.

The concept of incomplete SLE was proposed by Greer and Panush[29] who identified a group of patients in whom the ACR criteria for SLE were not met. These patients had an equivalent prevalence of locomotor and cutaneous symptoms, and a positive ANF but less frequently a malar rash, oral ulcers, serositis, haematological and neurological features and a lower requirement for steroids and immunosuppressants. There were also no renal or immunological disorders. Only 2 of the 38 later developed typical SLE. A follow up audit of 100 patients in the Scottish Highlands diagnosed by a single rheumatologist as having either fibromyalgia or no clear rheumatological disorder, showed that no patient was later diagnosed with SLE.

In the assessment of a patient with possible SLE, the differential diagnosis is wide. Conditions such as chronic fatigue syndrome, viral illness, depression, osteoarthritis and non-articular rheumatism are common, as are menopausal symptoms and hypochondriasis (the 'worried well'). These conditions need to be distinguished from other rheumatological disorders such as fibromyalgia, RA, Sjögren's syndrome and other connective tissue diseases, sarcoidosis and erythema nodosum. In hospital patients, malignant disease, infection and liver disease may present with fever and a high ESR, and thus also enter the differential diagnosis. Rarities must not be forgotten about lest they never be diagnosed.

ANF is a sensitive test with few false negatives which is good for screening. It, however, has a low specificity (~0.6) and therefore a high false positive rate of ~8% normals. If the test is carried out frequently and unselectively, it will produce many false positives. There are many diseases in which a positive ANF is seen including chronic active hepatitis (100%), scleroderma (90%), Sjögren's syndrome (80%), RA (60%) and diabetes mellitus (25%). The titre helps to differentiate between disease and non-disease, with a titre of $\geq$1:80 in 66% of SLE patients, 28% of RA patients and only 10% of patients with arteritis. Other tests such as anti-double stranded DNA antibody are useful but the specificity is diminished by the loss of sensitivity. Anti-Smith antibodies are too infrequently positive to be useful for screening. Hypocomplementaemia is often a useful indicator of SLE if the ANF is positive. Positive features in the history such as rash, serositis, and, on examination, arthritis, alopecia and rubs, may be balanced against negative, symptoms such as headache, fatigue and irritable bowel syndrome, and the absence of signs. Investigations such as the demonstration of an acute phase response, positive immunology in high titre, hypocomplementaemia and an active urinary sediment may support the clinical impression. Abnormal electrolytes, calcium or thyroid function support an alternative diagnosis.

As referrals are frequently test-driven, the history and examination should allow identification of multisystem symptoms and signs making a diagnosis of SLE probable or may direct thoughts towards alternative diagnoses. Laboratory tests and / or imaging may be required to confirm or exclude other diagnoses and specific immunological tests sometimes help to confirm or subclassify connective tissue disease. If the diagnosis is unclear avoid 'premature closure' and labelling the patient with an uncertain, potentially serious diagnosis. Keep the diagnosis open, continue active observation and review the case if new evidence arises.

In conclusion, ANF-positive referrals are common but SLE is rare. Positive and negative features on history and examination are most important in differential diagnosis. ACR criteria are not sensitive and often unhelpful in the outpatient clinic setting. Laboratory tests usually reinforce the clinical impression. If in doubt, consider alternative diagnoses, avoid 'premature closure' and maintain 'active observation'.

Positive anti-neutrophil cytoplasmic antibodies

The anti-neutrophil cytoplasmic antibody (ANCA) test, unlike ANF, is a relatively new development. ANCAs are antibodies against the cytoplasmic protein granules of neutrophils which contain enzymes involved in antimicrobial activity. As various different types of granules occur, then consequently several different types of ANCA are also found. The type of staining pattern seen indicates the type of ANCA. Indirect immunofluorescence identifies a cytoplasmic or cANCA pattern. If the cells are subjected to ethanol fixation, then a perinuclear or pANCA pattern is seen. Proteinase 3 (PR3) is the major antigen responsible for cANCA and myeloperoxidase (MPO) is usually the cause of a pANCA pattern, however the specificity of the ANCAs for the relevant antigens is low, and many other antigens can be responsible for either cANCA, pANCA or atypical staining patterns, the heterogeneity of pANCA being greater than that of cANCA. ANCAs are associated with systemic vasculitis but the exact inter-relationship is not clear, as ANCA negative vasculitis does occur. A mechanism that may be involved in the pathogenesis of vasculitis is the activation of neutrophils by ANCA to release PR3 with resulting damage to endothelial cells. Neutrophils can also express ANCA antigens on the cell surface.

Cytoplasmic ANCA is relatively closely associated with Wegener's granulomatosis (WG) but may also occur in microscopic polyangiitis (MPA) and renal limited vasculitis (RLV). However, diseases such as tuberculosis or other infections can cause a positive cANCA. The pANCA is more heterogeneous and occurs in approximately 45% of cases of MPA, but also in WG and other vasculitides e.g.

secondary vasculitis in RA or SLE. Other causes of a positive pANCA are Sjögren's syndrome, inflammatory bowel disease, infection and drugs. The presence of ANCA in RA is between 16-48%, but, on its own, a positive test is not useful to distinguish the presence of vasculitis in an individual patient. In fact the longer the duration of the RA, the more likely it is for the patient to have an ANCA and this may merely represent chronic auto-immune stimulation. Studies have been done to assess whether ANCA can be used to monitor disease and predict relapse. In a small study[30] asymptomatic patients were treated when their ANCA titres rose and relapses were prevented. A larger study showed ANCA titres were unrelated to disease activity,[31] although some authors have shown that the presence of ANCA (rather than the titre) is associated with an increased risk of relapse.[32]

ANCA standardisation was looked at in a large European multicentre study of vasculitis.[33] Of disease controls, 5% were cANCA positive and 20% pANCA positive by indirect immunofluorescence. If an enzyme-linked immunosorbent assay for PR3 and MPO was performed, the specificity was lowered but positive disease controls were still seen. Combining the tests eliminated specificity as a problem but at the expense of reduced sensitivity. In a group of patients with defined disease (RLV, MPA or WG) who were ANCA positive, the correlation between the type of ANCA and the disease was as expected with WG usually cANCA (PR3) positive, and MPA usually pANCA (MPO) positive, although there was a huge overlap. However, to meaningfully predict the diagnosis from the type of ANCA was difficult as, for example, the majority of patients with a positive cANCA had MPA rather than WG as MPA is more common. The pulmonary renal syndrome consists of renal impairment +/- haematuria or hypertension with associated pulmonary shadowing or haemoptysis. These patients have a high probability of having vasculitis, but other diagnoses such as SLE, infection, malignancy and drug reactions are included in the spectrum. Surprisingly, a study of 1,060 patients with the pulmonary renal syndrome showed only 13% were positive for ANCA.[34] In a small study in Edinburgh, 392 requests for ANCA were analysed by a hospital department with a positive rate between 0 and 22%. Indiscriminate ordering of tests needs to be discouraged and if the pre-test probability of a positive result is low then great care is needed to interpret the test result.

Clearly ANCA are associated with systemic vasculitis. As they are of a very heterogeneous nature, clinical judgement should be used in each case in the interpretation of a positive result, and the ANCA test should only be used in appropriate circumstances. Atypical ANCA have no clinical consequence at the current time. Since their introduction, the tests have been useful in raising awareness of the possibility of vasculitis as a diagnosis, but their use in monitoring is unclear. An ongoing trial is underway to assess whether alterations in ANCA titre predict disease relapse.

Positive antiphospholipid antibody

Antiphospholipid antibodies (aPL ab) may be associated with venous or arterial thrombosis. They have a broad spectrum of activity, some aPL ab bind to ß2 glycoprotein which binds to phospholipids, while others bind to glycoprotein-phospholipid complex directly. In addition, some aPL ab bind to the anticoagulant system, some interact with the endothelial system and some bind to platelets and may maintain them in the activated state. It is not known, however, how these antibodies produce the thrombotic effects. Antiphospholipid antibodies can be detected by demonstrating lupus anticoagulant activity with the Dilute Russell Vipers Venom test which is positive if prolonged and corrected by the addition of phospholipid. They can also be detected by immunological assays which can detect IgG, IgM and IgA anticardiolipin antibodies or antibodies to ß2 glycoprotein. Testing for IgA antibody is however probably not of additional value. If either of these tests is positive then they should be repeated after three months, as a positive result may occur temporarily with infection or inflammation.

There are various clinical syndromes associated with aPL ab. Primary antiphospholipid antibody syndrome occurs when these antibodies are present in association with venous or arterial thromboembolism, recurrent miscarriage or sterile endocarditis. In secondary antiphospholipid antibody syndrome, aPL ab are associated with thrombosis or recurrent miscarriage in the context of an underlying connective tissue disorder such as SLE, RA or Behçets disease. Antiphospholipid antibodies may also occur as an incidental finding with no history of thrombosis. This usually occurs in infections (human immunodeficiency virus, hepatitis C virus, syphilis, malaria), lymphoproliferative disorders, drugs (phenothiazines, quinine) and auto-immune conditions such as immune thrombocytopenic purpura; in these cases the presence of aPL ab is of no clinical significance. If aPL ab are present the likelihood of a clinical problem becoming manifest varies. The relative risk of developing thrombosis in those aged less than 40 years is 1.02. This rises to 1.72 in those with SLE. However, there is a statistically significant relative risk of 4.9 in those with a previous thrombosis. If the level of anticardiolipin antibody is very high (> 40 GPL), then the relative risk of thrombosis is also significant at 3.66. A study from St Thomas' in London in 1995 showed that the rate of recurrence of thrombosis is related to the anticardiolipin IgG titre.

The management of patients with aPL ab and thrombosis is controversial. Hughes *et al*[35] studied 147 patients retrospectively and showed that anticoagulation with high intensity warfarin (producing an INR > 3.0), with or without low dose aspirin, was significantly better at preventing recurrence of thrombosis over a decade than both low intensity warfarin (producing an INR < 3.0) with or without low dose aspirin and treatment with aspirin alone. However, there were 29 bleeding episodes, 7 of which were severe, out of 104 patients on warfarin, all of which occurred in those with an INR > 3.0. The management of recurrent fetal loss and antiphospholipid syndrome has been studied in 90 women who were randomised to receive aspirin alone or aspirin plus heparin 5000u bd. There was a statistically significant increase in the number of live births in those treated with aspirin plus heparin; in addition most of the miscarriages occurred in the first trimester and so it is important that treatment is started early.

In summary, patients should have an aPL ab test if they have had an arterial thrombosis, particularly if they do not have conventional risk factors such as smoking or atherosclerosis or if they have had a venous thrombosis at

a young age, because warfarin reduces the recurrence of thrombosis. Women with recurrent fetal loss (three miscarriages or more) should also have an aPL ab test because aspirin plus heparin results in more live births.

Pitfalls in bone density measurement

Osteoporosis is defined by the World Health Organisation as a disease characterised by low bone mass and a microarchitectural deterioration in bone tissue leading to enhanced bone fragility and hence an increased risk of subsequent fracture. Bone mineral density (BMD) measurement can be used to diagnose osteoporosis[36] and is as strong a predictor of osteoporotic fracture, especially of the hip, as blood pressure is for stroke, and much better than serum cholesterol for subsequent myocardial infarction. BMD cannot be used to diagnose fracture; this is done by plain radiography. In women, osteoporosis is defined as a BMD > 2.5 standard deviations below the young normal mean (T score). This arbitrary definition fits reasonably well with the pattern of disease expression seen in clinical practice as the prevalence of hip osteoporosis in women > 50 years of age (16%) is similar to that of hip fracture.

Peripheral and axial measurements of BMD do not give the same information but do give similar risk ratios for predicting fracture. Single or dual energy X-ray absorptiometry (DXA) has the advantage of directly measuring BMD but the disadvantage of using ionising radiation. Although the radiation dose delivered to the patient is negligible, appropriate radiation protection precautions must be taken by the staff working in the examination room. Peripheral BMD measurements can be used in the community to predict which patients to treat to prevent fracture. Quantitative ultrasound as a peripheral measurement has the advantage of requiring minimal training, involves no radiation and is portable. However, it does not measure BMD but some other as yet unspecified characteristic and hence cannot be used to diagnose osteoporosis.

The best site of analysis for determining BMD depends on which fracture you most wish to predict, e.g. hip BMD is better for predicting hip fracture than say lumbar spine fracture. Peripheral BMD measurements do predict hip and lumbar spine fracture, likewise quantitative ultrasound predicts Colles' and vertebral fracture. Measurement of hip BMD and ultrasound measurements at the heel together are a better predictor of fracture than either alone. All measurements predict fracture with similar strengths but site-specific measurements are the strongest predictor. However, the same is not true for BMD *per se*. In an individual patient, a peripheral BMD measurement cannot be used to predict BMD of the hip or spine, although it does predict fracture at these sites. Quantitative ultrasound has an even worse correlation for BMD even at the same site.

BMD can be used to monitor response to treatment. Monitoring relies on the knowledge of the precision of the technique. For DXA of the lumbar spine this is of the order of 0.8–1.5%, and at the hip of 1.5–2.5%. It is important to know if the change is real, i.e. what is the least significant change (95% CI), and also what is the expected annual change in response to treatment or disease at a specific site. The mean monitoring interval must also be known. DXA can be used to monitor osteoporosis at the lumbar spine at an interval of two years.

As a lumbar spine DXA scan is performed over L1-L4, this measurement is not of value in the elderly as various factors such as marginal osteophytes or facet joint osteoarthritis, secondary osetoarthritis from scoliosis, aortic calcification and vertebral fractures, all lead to a falsely high BMD measurement at this site. As the rate of loss of bone varies at different ages at different sites with loss in the lumbar spine > hip > heel, a universal T score for peripheral and axial sites is not suitable. Likewise, a universal treatment for osteoporosis based on a single site measurement is also incorrect.

In conclusion, BMD measurements can be used to diagnose osteoporosis as defined at present. Peripheral (forearm or heel) and axial (spine or hip) measurements do not give the same information but do give similar risk ratios for predicting fracture. BMD measurements can be used to monitor treatment of osteoporosis at the lumbar spine after two years, but are not of use in the elderly.

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