Autoimmune diseases and infections: controversial issues

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ABSTRACT
The etiology and pathogenesis of certain types of disease remain controversial and stand like a bridge that crosses infectious, autoimmune and autoinflammatory pathways. Infection, for example, may initiate a disease, although it is the genetic regulation in the host, the interplay between virus or bacteria persistence and autoimmunity that produces the later phases of disease, the antigenic determinants responsible for inducing autoimmune disease, and the pathogenetic effector mechanisms. Infections agents cause pericarditis, but in 85% of cases it is “idiopathic”. It has also been shown that persistent Clamydia pneumoniae, Porphyromonas gingivalis, and Helicobacter pylori infections cause host immunity and promote atherogenesis. A number of infectious agents have been suggested as potential triggers for primary biliary cirrhosis. Infections and vaccinations have also been linked to the pathogenesis of fibromyalgia syndrome, a common, chronic syndrome of widespread pain. Many factors are also responsible for fever of unknown origin such as: infections, autoimmunity disease, etc. However, it is difficult to determine a direct correlation between the infections agents in such a large group of diseases. The aim of this review is to analyze some of the controversies about the role of infections in autoimmune diseases.

Introduction
The etiology and pathogenesis of autoimmune diseases and the roles played by infections highlighted a number of controversial issues. Pericarditis can be caused by different factors including infections or autoimmunity. Sometimes the etiology cannot be identified by means of standard laboratory techniques and so it remains “idiopathic” in approximately 85% of cases.

Atherosclerosis is a chronic arterial disease that is initiated and perpetuated by pro-inflammatory lipid abnormalities. Experimental evidence supports the idea that autoimmunity contributes to atherosclerosis by means of autoantibody-mediated pro-atherogenic mechanisms, as seen in systemic lupus erythematosus and antiphospholipid syndrome. It has also been shown that persistent Clamydia pneumoniae, Porphyromonas gingivalis, and Helicobacter pylori infections cause host immunity that promotes atherogenesis.

Primary biliary cirrhosis (PBC) is probably the result of a susceptible genetic background along with infectious trigger, leading to tolerance breakdown. A number of infectious agents have been suggested as potential triggers, but there is no definite evidence based on epidemiological and experimental data. Fibromyalgia syndrome (FMS) is a common, chronic syndrome of widespread pain that mainly affects women. Its etiology is not entirely clear, but infections and vaccinations have both been linked to its pathogenesis. Recent studies have noted an association between FMS and active hepatitis C-B virus infections, as well as between various vaccinations and symptom complexes that include FMS and chronic fatigue syndrome.

Fever is a common symptom in patients with either infections or autoimmune diseases. In some cases it can be difficult to identify the cause of such fever and a diagnosis of fever of unknown origin (FUO) is given. FUO was defined as fever higher than 38.3°C (101°F) on several occasions, persisting without a specific diagnosis for at least three weeks after appropriate investigations. Recently, the spectrum of illnesses now considered to cause FUO in the immunocompetent host has changed as a result of a number of factors including changes in demographics and the availability of new diagnostic techniques.
**Pericarditis: infectious or autoimmune?**

Acute pericarditis accounts for 5% of presentations at Emergency Departments due to non-acute myocardial infarction (1). It is an intensely inflammatory disease, which recurs in 20–40% of patients (2), and is characterised by chest pain, pericardial rub, widespread saddle-shaped or concave upward ST segment elevation at electrocardiography, pericardial effusion, fever, a high erythrocyte sedimentation rate (ESR) and high C-reactive protein levels (CRP). It does not include other conditions such as symptomatic pericardial effusion with normal CRP, or asymptomatic pericardial effusion.

**Etiology**

Anything may cause pericarditis, and an etiological search is often inconclusive in clinical practice. Using a systematic battery of serological tests Levy et al. (3) identified an infectious etiology in approximately 20% of cases. The most frequently involved viruses were Echovirus and Coxackievirus, with the rare involvement influenza, EBV, adenovirus, varicella, rubella, mumps, HBV, HCV, HIV, parvovirus B19 and human herpes virus 6. The most common bacteria were *Mycobacterium tuberculosis* (4-5%) and *Coxiella burnetii* (5-7%), with the rare involvement of *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus*, *Staphylococcus*, *Chlamydia*, *Mycoplasma*, *Legionella*, *Leptospira* and *Listeria*. *Histoplasma fungi* were more likely in immunocompetent patients, and *Aspergillus*, *Blastomyces* and *Candida* more likely in the immunosuppressed; a parasitic etiology was very rare and involved *Echinococcus* or *Toxoplasma*. European investigators have conducted extensive studies based on pericardioscopy, multiple epicardial biopsies, and polymerase chain reaction (PCR), and reported a higher prevalence of infection or reinfection than other investigators, particularly viral (Coxackie A9, B1-4, Echo 8, mumps, EBV, CMV, varicella, rubella, HIV, Parvo B19); bacterial (Pneumococcus, Meningococcus, Gonococcus, Hemophilus, Treponema pallidum, Borrelia, Chlamydia, Tuberculosis); fungal (Candida, Histoplasma) and parasitic infections (Entameba histolytica, Echinococcus, Toxoplasma) (4). On the other hand, some authors suggest that such intensive etiological searches may not be warranted as they do not affect therapy or prognosis in most cases (5). An infectious or systemic etiology cannot usually be identified using standard laboratory techniques without molecular tests and, in the real world, the most common specific etiologies reported in published clinical series are tuberculosis (TB) 5% (3.9-4.7%), neoplastic pericarditis 5% (4.7-7%), rheumatic autoimmune diseases 5% (1.7-10.2%); idiopathic and/or viral acute pericarditis are found in 80-90% of the cases occurring in immunocompetent patients from developed countries (6). Concerning the diagnosis of TB, it must be stressed that pericardial fluid cultures are positive in only 50% of cases, whereas the sensitivity and specificity of pericardial fluid adenosine deaminase values of >30 u/L are 94% and 68%, and those of interferon-γ (IFN) values of >200 pg/L are both 100% (7).

**Pathogenesis**

Viral pericarditis is the most common infection of the pericardium. The inflammatory abnormalities are due to direct viral attack, the immune response (antiviral or anticardiac) or both. As early viral replication in pericardial and epicardial tissue elicits cellular and humoral immune responses, pericarditis starting as an infection becomes an autoimmune disease (8).

Recurrent pericarditis is often considered to be an autoimmune condition. The evidence for this is the presence of pro-inflammatory cytokines such as IL-6, IL-8 and INF-γ in pericardial fluid (their absence in plasma suggests a local inflammatory reaction); the prevalence of autoantibodies; the occurrence of new autoimmune diagnoses; and the good response to anti-inflammatory or immunosuppressive therapy. We have recently tried to elucidate some of the pathogenically relevant aspects of this condition (9). Together with the Padua and Turin group, we tested 100 consecutive patients with idiopathic recurrent acute pericarditis (IRAP), and 122 healthy volunteers for the presence of anti-nuclear antibodies (ANA), which were detected in 41% of the patients and in 9% of the healthy volunteers (p<0.001). Low titres (1/40-1/80) were found in the majority of cases; no high concentrations (1/640) were recorded. The follow-up rates of complication and new diagnoses were similar in the patients with or without ANAs. These findings suggest a possible autoimmune pathogenesis, but they are often clinically non-specific.

During the follow-up of another study involving 46 IRAP patients, we made new diagnoses of primary Sjögren’s syndrome in four cases (8.7%) and of rheumatoid arthritis (ACR criteria) in one (2.2%). The findings of the Turin group were similar: only autoimmune conditions were diagnosed during the very long follow-up, including Horton’s arteritis, polymyalgia rheumatica and Crohn’s disease.

Finally, we assessed the role of multidrug “rheumatological” therapy, and found that a protocol including the very slow tapering of corticosteroids (over months, as in the case of many rheumatological conditions), the use of non-steroidal anti-inflammatory agents (NSAIDs) at the recommended doses, and colchicine (if tolerated) can be very useful in controlling the activity of IRAP. The improvement was more marked and faster when colchicine was used.

On the other hand, on the basis of their good response to colchicine, it has been suggested that some IRAP patients may have an atypical or sub-clinical form of familial Mediterranean fever (FMF), and we have also shown that IRAP may be familial. We have not found the mutations associated with FMF, but another underlying auto-inflammatory condition remains possible (10).

**Autoimmunity, infection and atherosclerosis**

Atherosclerosis is a chronic inflammatory disease of the arterial wall associated with various risk factors that promote plaque and thrombus formation. One typical feature of atherosclerotic
lesions is the accumulation of lipid-laden foam cells in the arterial intima, and the oxidation of low-density lipoprotein (LDL) triggers the generation of a series of by-products that contribute to the initiation and progression of atherosclerosis.

Antiphospholipid syndrome (APS) is an autoimmune disease characterised by the presence of anti-phospholipid antibodies (aPLs), associated with arterial or venous thrombosis, or pregnancy morbidity. It is now generally accepted that clinically relevant aPLs recognise the phospholipids binding plasma proteins such as β2-glycoprotein I (β2GPI) (11-13), which also binds to oxidized LDL (oxLDL), and oxLDL/β2GPI complexes have been identified as atherogenic autoantigens (13-16).

Circulating oxLDL/β2GPI complexes have been detected in the serum of patients with chronic inflammatory and systemic autoimmune diseases associated with atherosclerotic complications. Anti-β2GPI antibodies reacting with oxLDL/β2GPI complexes are also frequent in systemic lupus erythematosus (SLE) and APS. The in vitro macrophage uptake of oxLDL/β2GPI complexes is significantly increased in the presence of IgG anti-β2GPI antibodies (13-15), which strongly suggests that some APS-derived anti-β2GPI antibodies are pro-atherogenic.

Our own recent studies have elucidated the macrophage intracellular trafficking of β2GPI complexed with lipid vesicles (17). β2GPI complexed with oxLDL or phosphatidyserine (PS) liposomes can be transported into lysosomes and activate β2GPI-specific T cells. In addition, antigen presentation to T cells is further facilitated by the presence of IgG anti-β2GPI antibodies (18).

It has been suggested that the humoral “autoimmune” response induced by chronic infections is associated with cardiovascular disease (CVD). Ayada et al. have recently demonstrated that Helicobacter pylori infection accelerates atherosclerosis via the Th1-dominant response to H. pylori-derived heat shock protein 60 (Hp-HSP60) in atherosclerosis prone mice (19).

**Oxidative modifications of LDL and its atherogenic complexes**

Circulating LDLs (consisting of free and ester forms of cholesterol, phospholipids and triglycerides) and apolipoprotein B100 (apoB100) pass into the sub-endothelial space, the presumed site of LDL oxidation in vivo. LDLs are frequently exposed to cell-derived oxidants, which modify them and apoB100. Polyunsaturated fatty acids, phospholipids and triglycerides also undergo free radical-initiated oxidation, which leads to a broad range of smaller fragments, including aldehydes and ketones that can become conjugated to amino lipids or apoB100. These fragments further participate in chain reactions that propagate and amplify the damage.

β2GPI, a major target antigen for aPLs, binds to negatively charged molecules, such as cardiolipin, PS and oxLDL. It is assumed that the β2GPI ligands derived from LDL oxidation are oxidised by-products of cholesterol linoleate, the major cholesteryl ester: e.g., 7-ketocholesteryl-9-carboxynonanoate (9-oxo-9-(7-ketocholest-5-en-3β-yloxy) nonanoic acid) (oxLig-1). The ω-carboxyl function of the acyl chain, and the ketone function at position 7 of the cholesterol backbone in the ligands, are necessary for high-affinity interactions with β2GPI, and stable oxLDL/β2GPI complexes are frequently detected in the serum of anti-immune and/or atherosclerotic patients with APS, SLE, type 2 diabetes mellitus (DM), CVD, etc.

CRP is an acute phase protein that is synthesised in the liver, but a number of recent reports have suggested that it is also produced in atherosclerotic lesions. We have shown that CRP can form complexes with both oxLDL and β2GPI in atherosclerotic lesions, and that these are released into the circulation (16). CRP/oxLDL/β2GPI complexes have been detected in DM patients with atherosclerosis.

Increased cardiovascular mortality due to the accelerated development of atherosclerosis has been reported in patients with SLE and APS, and these findings have generated a great deal of research into the role of autoimmune mechanisms in atherogenesis. APS is the most common cause of acquired hypercoagulability in the general population. The presence of aPLs significantly increases the risk of thrombosis in autoimmune diseases, and autoimmune vascular inflammation may cause LDL oxidation and promote the interaction of oxLDL with β2GPI. The majority of anti-β2GPI antibodies react with oxLDL/β2GPI complexes, thus providing initial and direct clues that aPLs participate in atherosclerosis. β2GPI co-localises with oxLDL, immunoreactive CD4+CD8+ T cells and immunoglobulins in human and animal atherosclerotic lesions, and these findings further support the hypothesis that β2GPI and anti-β2GPI antibodies, as well as oxLDL, play a pathogenic role in the development of arterial thrombosis (atherothrombosis) in SLE/APS patients.

β2GPI is preferentially taken up by macrophages when complexed with oxLDL or PS liposomes via scavenger receptors, after which it was finally transported into lysosomes (17). The binding of β2GPI to oxLDL or PS liposomes facilitates the presentation of β2GPI epitopes by macrophages to autoreactive T cells. The formation of IgG immune complexes enhances the activation of T cells (18). Interestingly, the in vitro stimulation of macrophages by β2GPI/oxLDL complexes and anti-β2GPI antibodies leads to the membrane expression of CD36 and FcγRI (17). β2GPI/oxLDL complexes therefore represent a major atherogenic autoantigen, and IgG anti-β2GPI auto-antibodies may facilitate both antigen presentation and foam cell formation in SLE/APS.

**Chronic infection and atherosclerosis**

A number of epidemiological studies have suggested that the immune responses to persistent pathogen infections probably cause or promote atherosclerosis. HSP60 is a ubiquitous and highly conserved molecular chaperone expressed in both eukaryotic and prokaryotic organisms, and it seems that immune responses against infection-derived HSP60 are among the key pathogenic processes that misdirect endogenous host HSP60 on the endothelium in early atherosclerosis. Recent studies have indicated that some...
putative immunogenetic epitopes in HSP60s are associated with atherogenesis, and that they could be useful for diagnosis and/or treatment. Okada et al. (20) have recently demonstrated that IgG antibodies against a particular amino acid sequence of Hp-HSP60 can be detected by enzyme-linked immunosorbent assay (ELISA) using a solid phase of full-sequenced human counterpart, and that these antibodies are independent diagnostic markers relevant to cardiovascular disease. A particular region in the HSP60 sequence (Glu\textsuperscript{141}, Leu\textsuperscript{146}) is the predominant epitope associated with CVD.

Immune responses against HSP60 of pathogenic origin are thought to be defensive events that are misdirected to a human counterpart as a result of molecular mimicry. It is still unclear whether such antibodies are pathogenic, but it can be said that we can serologically predict atherosclerosis-induced cardiovascular disease by detecting anti-HSP60 antibodies.

The pathogenic role of HSP60-reactive T cells is the subject of intensive research. Recent studies have shown that the Th1 differentiation of naïve T lymphocytes contributes to atherogenesis, and that HSP60 cell immunity blockade is more important than innate immune modulation. In this context, we have shown that H. pylori infection induces atherosclerosis in apo\textsuperscript{−/−}, LDL\textsuperscript{−/−} mice (19) (in preparation). Hp-HSP60-specific T helper 1 (Th1)-dominant immune responses play a major role in the progression of atherosclerosis, and probably cause misdirected autoimmune against the endogenous HSP60 expressed on the stressed cells of vascular endothelium due to molecular mimicry. Appropriate treatment with antibiotics or immunotherapy directed against HSP60 (which regulates Th1 induction) may therefore sufficiently reduce the progression of atherosclerosis.

Unravelling the roles of the newly discovered elements and mechanisms contributing to the initiation and progression of atherosclerotic disease brings us closer to being able to develop more effective diagnostic, therapeutic and preventive measures aimed at reducing or (optimistically) eliminating the burden of CVD.

Environmental clues in primary biliary cirrhosis

What causes the breakdown of immunity in genetically susceptible individuals is poorly understood (21). It has been demonstrated that infectious agents (particularly bacteria) can induce autoimmune responses in experimental settings, and molecular mimicry is the most widely studied mechanism explaining these observations (22). The idea that infectious triggers may be involved in primary biliary cirrhosis (PBC) comes from epidemiological observations of comorbidities and risk factors (23).

A number of bacterial strains have been proposed as causing PBC as a result of molecular mimicry (24), but most of the collected evidence relates to Escherichia coli and is mainly based on reports of an increased prevalence of urinary tract infections in patients with PBC (23). Nevertheless, on the basis of serum cross-reactivity, the other infectious agents include Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Salmonella minnesota, Mycobacterium gordonae, Neisseria meningitidis, and Trypanosoma brucei. The common commensal yeast, Saccharomyces cerevisiae, has also been recently investigated because of the expression of antimitochondrial (AMA) antigens in extra-mitochondrial sites, but serological studies indicated that the reactivity against the yeast (ASCA) was not disease specific. Interestingly, contrasting evidence has been collected concerning the role of Chlamydia pneumoniae in the pathogenesis of PBC.

Finally, recent serological data from Selmi et al. suggest that a ubiquitous xenobiotic-metabolising Gram-negative bacterium, Novosphingobium aromativorans, is the best candidate cause of the induction of PBC because it elicits a specific antibody reaction (estimated to be 100-1,000 times greater than that against E. coli) and its specific 16S rRNA sequences have been detected in human fecal samples (25). To a certain extent in conflict with current theories concerning the relationship between infections and autoimmunity (26), the new hypothesis is that the microorganism enters the human system through the digestive mucosa and, at this point, bacterial mimics containing lipidic acid residues might be modified by xenobiotics to form immunoreactive adducts. This modification may be sufficient to trigger the innate immune system to initiate a cascade of local inflammatory events (e.g., via Toll-like receptors (TLRs)), thus leading to local dendritic cell activation and antigen processing. Mucosal APCs then activate autoreactive T and B cells that are directed towards the liver through the portal system: the T cells participate directly in the autoimmune injury and/or further recruit autoreactive lymphocytes, whereas the B cells secrete AMAs, particularly of the IgA type. The IgA AMAs are then transported to the vascular side of the bile duct cell where they react with the pyruvate dehydrogenase complex (PDC)-E2-like molecules located on the luminal surface cell membrane. This binding then initiates the apoptotic signalling cascade. Ultimately, the immune complexes of post-apoptotic PDC-E2 and IgG AMA, and the direct cytopathic effects of autoreactive T cells (and possibly AMAs), contribute to the tissue injury observed in PBC.

For the sake of completeness, Selmi et al. (27) also note that a novel human beta-retrovirus has been identified in lymph nodes and other samples from patients with PBC, thus suggesting that this mouse mammary tumor virus (MMTV)-like virus might play a role in the pathogenesis of PBC. However, our laboratory has failed to confirm this hypothesis using a different molecular and immunological approach to a large series of patients and controls, which argues against using any anti-retroviral therapy in PBC.

Xenobiotics are foreign compounds that may alter or complex with defined self- or non-self proteins, thus changing the molecular structure of the native protein in such a way as to induce an immune response (28). This may then lead to the cross-recognition of the self-protein, which could in turn perpetuate the immune response and give rise to chronic autoimmunity (29).

It has been suggested that a number of specific compounds may play a role in Rheumatic diseases and infections / P. Baio et al.
various autoimmune conditions. Interestingly, most xenobiotics are metabolised in the liver, thus increasing the potential for liver-specific protein alterations. Experiments have shown that specific organic structures attached to mitochondrial antigens are recognised by serum from PBC patients with greater affinity than the native forms of the same antigens (30). These findings were the first to indicate that an organic compound may serve as a mimotope for an autoantigen, thus further supporting the existence of a potential mechanism by means of which environmental organic compounds may cause PBC. For example, it has been found that some halogenated compound can induce AMA production in animal models, and that the AMA-positivity was reversible once the immunisation ceased.

It is worth noting that the vast majority of the data concerning molecular mimicry in PBC have been obtained by studying humoral immunity (i.e., AMAs) in patient serum or animal models, whereas cell autoimmunity has been less widely studied. For these reasons, the role of xenobiotics and infectious agents in the onset of PBC needs to be further investigated by means of the development of an appropriate animal model and detailed epidemiological studies aimed at ascertaining exposure to specific environmental factors.

**Fibromyalgia, infections and vaccinations**

Fibromyalgia syndrome (FMS) is a common, chronic syndrome of widespread pain that mainly affects women. Although its etiology is not completely understood, various neuroendocrine disturbances and abnormalities in autonomic function, as well as genetic factors, have been implicated in its pathogenesis (31). Infections and vaccinations have also been linked to the pathogenesis of FMS (32). Adak et al. (33) found that carrying chronic hepatitis B seems to increase the risk of FMS and many of its typical symptoms, and an association with active hepatitis C virus (HCV) infection has been reported in some patients, although it was not associated with liver damage or autoimmune markers (34). Similarity, Buskila et al. (35) have reported a high prevalence of FMS (16%) in HCV-infected patients, especially women, and suggested that recognising FMS in patients with HCV should prevent the misinterpretation of FMS symptoms as part of the liver disease and enable physicians to reassure the patient and alleviate the symptoms themselves.

It has also been reported that FMS is a common cause of musculoskeletal symptoms in HIV-infected patients (36). Buskila et al. (37) found that 15 out of 51 patients with HIV (29%) met the criteria for fibromyalgia, which was significantly associated with myalgia and arthralgia, but not with age, the duration of HIV infection, the stage of HIV disease, or zidovudine therapy.

It is not clear how viruses might trigger FMS, but central nervous system cytokine activation via viral neurotranspism and subsequent glial activation may play a role (38).

Associations have been described between various vaccinations and symptom complexes including FMS and chronic fatigue syndrome (CFS). The US National Vaccine Injury Compensation Program and Court of Federal Claims have accepted a causal relationship between some chronic arthropathies (including cases of FMS) that appeared between one and six weeks after the administration of rubella vaccine (39).

Gulf War syndrome is a functional multi-system entity that shares many of the clinical characteristics of FMS. Hootopf et al. (40) reported that although the administration of multiple vaccinations before deployment in the Gulf did not appear to be harmful in themselves, the combination of their administration and the stress associated with being stationed in a combat zone (and possible other factors) may increase the risk of developing ongoing ill-health.

Further well-designed and controlled studies are needed to clarify the possible role of infection and vaccination in the pathogenesis of FMS.

**Fever of unknown origin: when it is infectious or when not**

Fever of unknown origin (FUO) was defined as fever higher than 38.3°C (101 F) on several occasions, persisting without a diagnosis for at least 3 weeks after certain appropriate investigations. Despite immunocompromised patients and those with nosocomial FUO have been excluded because these patients require different diagnostic approaches (41), classic FUO continues to be one of the most diagnostic challenges in medicine (42). Reported causes of FUO exceed 200, and fall into four diagnostic categories including infections, tumors, non-infectious inflammatory diseases (NIID), miscellaneous, and undiagnosed illnesses. The relative frequencies of diagnoses within these 5 categories vary from series to series, depending on the era in which the series was published, ages of patients, and geographic area. We reviewed the literature on this topic, especially focusing on epidemiologic and diagnostic features. Even though the accuracy of clinical investigations has improved in recent years, an overview over the last 5 decades of the largest FUO series shows a clear trend toward an increasing percentage of unexplained cases (43, 44). This finding can at least partially be explained by the changing case definition over time. Indeed, in more recent series the exploratory phase before a prolonged unexplained fever would qualify as FUO was reduced from 1 week to 3 days (41). Other authors have attributed the rising incidence of undiagnosed cases to the introduction of new diagnostic techniques which may allow to obtain a diagnosis before the FUO criteria are met. In addition, the high percentage of patients with periodic or episodic fever (febrile periods separated by symptom-free intervals of variable duration but lasting at least 2 weeks) is known to be an important factor explaining the high number of undiagnosed cases (43-45). Infections that may cause periodic fever such as prostatitis, cholangitis, infective endocarditis, and spondylitis are easily ruled out by appropriate diagnostic investigations. Colon cancer, lymphoma and other haematological disorders represent malignancies that should be actively looked for. In addition, all connective tissue diseases and the so-called hereditary periodic fever syndromes (i.e., Familial Mediterranean Fever) can flare up suddenly and remit spontaneously, producing a recurrent...
fever (45-47). The prognosis of most of patients (74%) with unexplained FUO after following the adoption of a standardized diagnostic protocol was found to be excellent in a prospective study of 167 patients (44). Similarly, among 61 individuals with undiagnosed FUO that were followed up at least 5 years or until death, most cases have been found to resolve spontaneously and the mortality rate was only 3.2% (48). In a more recent prospective study, the proportion of undiagnosed cases (51%) has shown to be high even after following a structured diagnostic protocol (49). This finding has been attributed to a number of factors including patient referring after extensive investigations elsewhere, adoption of strict diagnostic definitions, and, again, a high percentage of patients with periodic fever. Although infection was found to be the most common cause of FUO, accounting for 23.0 to 39.5% of cases (42), the proportion of patients that are eventually diagnosed with infection have decreased in recent years. In a study carried out in the Netherlands (49), infection (i.e., chronic persistent persinsis, osteomyelitis, and bronchiectasia/pneumonia) was found to be the cause of FUO in 16% of cases. A similar percentage of patients diagnosed with infection was shown in other studies performed in northwestern Europe (43, 44). On the other hand, in a study from Turkey, infections accounts for a much larger percentage of cases (34%) which can be explained by the high incidence of tuberculosis (50). Indeed, this may occur in other European countries with heavy representation of immigrants coming from areas where tuberculosis is hyperendemic. In the elderly connective tissue diseases (i.e., temporal arthritis, polymyalgia rheumatica) surpas even infections as the leading cause of FUO. In patients older than 65 years in whom an infection is identified, intra-abdominal abscesses, complicated urinary tract infections, extrapulmonary tuberculosis, and infective endocarditis have been found to be the most common causes of FUO (51). Although relatively few cases of FUO go undiagnosed in elderly patients, elderly FUO patients have a substantial poorer prognosis than young patients because of the higher incidence of malignancies (52). As a whole, since a significant proportion of all FUO cases remain undiagnosed, there is no diagnostic gold standard against which diagnostic tests may be measured to find out diagnostic yield. Although several experience-based recommendations for the diagnostic workup of FUO exist, a uniform diagnostic algorithm may be not useful because investigations are most helpful when applied in a guided fashion determined by each individual case and potential diagnostic clues (PDCs) (42). PDCs were defined as all localizing signs, symptoms, and abnormalities potentially pointing towards a diagnosis. However, an important problem is the increasing frequency of misleading PDCs, of up to 81% in most recent series (44, 49). Non-invasive laboratory tests including serologic tests for microbial pathogens and for various rheumatologic disorders yield the diagnosis of FUO in approximately a quarter of the cases. Imaging techniques such as [18F]fluorodeoxyglucose (FDG) positron emission tomography (PET) scan have been shown to be useful in the evaluation of FUO. In a comparative study of 58 consecutive cases of FUO, FDG-PET scan and gallium scintigraphy were found to yield diagnostic information in 35% and 25% of these cases, respectively; in addition, FDG-PET scan compared favourably with gallium scintigraphy because of more quick results, within days instead of days (53). Similarly, in a recent prospective study FDC-PET scan has been shown to be helpful in 33% of 73 patients, contributing significantly more often to the final diagnosis in patients with continuous fever than in those with periodic fever (47). In conclusion, the spectrum of illnesses now considered to cause FUO in the immunocompetent host has changed as a result of a number of factors including changes in demographics and the availability of new diagnostic techniques. In western countries the proportion of cases caused by infections and neoplasms has decreased whereas that caused by NIID and undiagnosed conditions has risen significantly. This should inspire further researches to clarify underlying pathophysiological mechanisms.

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S-80

Rheumatic diseases and infections / P. Bai et al.