AMERICAN UNIVERSITY OF ARMENIA
COLLEGE OF PUBLIC HEALTH

PROJECT PROPOSAL: "Assessment of Susceptibility Risk Factors and Course Predictors at Seronegative Spondyloarthropathies (SPA)"

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PROJECT NARRATIVE

1 *INTRODUCTION*

There are about ten overlapping and mutually transforming inflammatory axial and peripheral joint arthropathies where combinations of extraarticular manifestations like inflammatory bowel diseases (IBD), mucosa, skin and nail lesions, cardiovascular, lung, genito-urinary and ocular inflammatory disorders also might be occurred. In addition, familial aggregation, greater than expected occurrence of HLA-B27 immunomarker, as well as certain types of gut and genito-urinary habituated pathogens (and strong association between the outbreaks caused by them and arthritis); absence of IgM rheumatoid factor, assume possible similar etiopathogenesis of spondyloarthropathies (SPA) and combine them into the common group ( ).

Their Public Health relevance based upon world-wide significantly high cumulative frequency (up to one per cent which might be increased along with the increasing STD and IBD morbidity and contagious enteropathy outbreaks) as well as an early onset of impairment and disability among active labor and military population. ( ) Moreover, often recurred arthritic attacks of SPA patients are the major cause of temporal disablement. ( )

However, certain entities within SPA group are unequal regarding their prognostic value, i. e. some of them have self-limited course, others are initially indetermined for early disability onset. Moreover, among this group there are interrelated conditions (especially at the onset of disease) do not meet the diagnostic criteria for any of them (so called undifferentiated SPA), but 20 per cent of which might be transformed to Ankylosing Spondylitis (AS) with poor prognosis. ( )

Assuming, that specific phenotyping expression of certain SPA entities is the end product of variety of interrelating genetic and environmental factors there is an urgent need to find out which of them are most relevant or amenable for poor prognosis and are there any preventable / curable ones among these potential course predictors.

II OBJECTIVES

The objectives of this project are:

- to conduct long term prospective follow-up study concerning the SPA-morbidity, disability and mortality among families, where there is at least one patient with definitive SPA.

- to organise surveillance system (arthritis registry) based upon the existing city rheumatological facilities for systematic and comprehensive data collection about SPA.

- to seek for the potential risk factors of SPA susceptibility and disability (especially preventable/curable in patients with undifferentiated SPA, who are first degree relatives (FDR) of patients with different SPA forms.

- to reveal predictors for certain types of SPA developed from initially undifferentiated SPA (intermediate or process markers).

- to evaluate them as the additional predictors of early disability.

- to investigate the dynamics of “exposure-outcome onset” relationship over the follow-up period (5-8 years).

III. THE HYPOTHESIS to be tested is whether there are course predictors (particularly preventable or curable) among the huge group of baseline variables of SPA patients; are there any interaction among them in terms of the development of certain adverse outcomes.

IV. BACKGROUND AND JUSTIFICATION.

ASSESSMENT OF SUSCEPTIBILITY RISK FACTORS AT SPA.

Data obtained due to descriptive epidemiological approach delineate not only the magnitude and PH relevance of the problem investigated, but also uncover risk factors in disease causation if the occurrence of disease consider in terms of demographic, genetic and environmental influences. Additionally, epidemiological method is being applied to the study of natural history of disease and those factors,that impact on the prognosis. Concerning SPA, there is well established evidence of early onset (less than 40 years old). Moreover juvenile onset SPA in native North American population accounts for at list half of arthritides in childhood. ( ) At the same time, SPA are an only exception (excluding gout) among the Rheumatic diseases where there is a striking male excess (even after age adjustment). Moreover the SPA course in females generally is less complicated and prognosis is usually benign. This is the major reason of underestimation of SPA morbidity among them. ( )

Such an excess is a useful starting point for considering etiologic hypothesis and the effect of hormonal and reproductive factors. ( )

The relative frequencies of the SPA subtypes vary within and between racial groups. For example, Ankylosing Spondylitis (AS) is a predominant SPA form in Caucasians of Northern European descents (a peak over two per cent in Northern Norway). The highest prevalence of AS is also determined among Haida and Pima North American Indian male population (27-60 per 1000) ( ) whereas in American Blacks the same index is equal only 1/4 of Caucasians and extremely rare in African Blacks. ( ) For indigenous Polynesians and Islanders of Papua-New Guinea as well as Japanese population AS is significantly lower and is not known in Australian aboriginal race. ( )

Racial and geographic differences revealed in SPA morbidity can also be useful in framing hypotheses assuming the presence of genetic and environmental peculiarities among the population of interest.

The next evidence in favour of genetic background of SPA, which permits to consider it as a possible risk factor is **familial aggregation or clustering**. Prevalence of "definite" and especially "possible" forms of different SPA is 20-30 times higher in SPA families vs general population, and commonly the relatives of probands with certain SPA subtypes copy the same form of disease. ( ) In most up to date literature reviews it was been found that studies of twins generally supported a genetic predisposition of SPA, since concordance was usually greater (> 50%) among MZ twins than in DZ twins. ( )

The search for an explanation of this **increased SPA heritability** lead to the discovery of the strong association between SPA morbidity and the occurrence of the immunogenetic marker HLA-B27 (one of the antigens of Major Hystocompatibility Complex MHC allocated in 6 chromosome). ( ) There is an increased prevalence of HLA-B27 (up to 90-95 per cent) among SPA patients which varies according to the frequency of certain SPA subtype. ( ) Moreover, negative predictive value of HLA-B27 testing is also exceptionally high suggesting negligible chance to posses SPA for HLA-B27 (-) individuals. ( ) HLA-B27 (+) SPA patients are more likely develop more prolonged disease with grater potential for chronicity ( ) as well as for increased frequency of spinal involvement and systemic manifestations like fever, uveitis, balanitis, oral or/and genital ulcerations. ( )

But at the same time, the chance of HLA-B27 (+) persons to contract SPA is only 2-8% suggesting much more sophisticated mechanisms of disease susceptibility. One of the possible explanations of this phenomenon might be related to highly polymorphic structure of HLA-B27 molecule. Indeed, recent epidemiological studies have revealed the different patterns of eleven HLA-B27 subtypes distribution among different ethnic populations as well as among AS patients and healthy controls. Thus, data of transracial gene mapping of HLA-B27 subtypes have shown that HLA-B2705 and B2702 were characteristic for Caucasians (HLA- B2705- predominantly for circumpolar and subarctic population, HLA-B2702 - Middle East and North Africa groups: Jews-Arab-Berbers); HLA-B2704 or HLA-B2707 were found exclusively in Asians (Orientals) and HLA-B2703 was overrepresented in West Africa. An association with AS was ascertained for HLA-B27 02, 04, 05, 07 alleles (predominantly for 05, 04 and 02). No data have been reported regarding association of AS and HLA-B2701, 03, 06, 09, 10 and 11. Moreover HLA-B2703, 06 and 09 have certain protective value in terms to contract AS for some population and not for other. For example, in this sense HLA-B2706 and 09 subtypes are absent in AS patient groups of the Thai, Indonesian population (Malaya's) and Sardinians (only for HLA-B2709) and contrary HLA-B2706 was found in two Chines AS patients. HLA-B2703 is the predominant subtype in West Africa (Senegal, Gambia) where SPA is exclusively rare. However, HLA-B27 (+) three AS patients have recently been found in this population. At the same time no AS case at all was seen in the Fula ethnic group of Gambia where HLA-B27 prevalence was equal six per cent

(HLA-B2703 - 32% and HLA-B2705 - 68%). This data suggest the existence of some non B27 derived protective factors reducing the AS prevalence in study population ( ).

In Asian Indians four HLA-B27 subtypes were encountered (02, 04, 05, 07). HLA-B2704 (common Oriental subtype) and HLA-B2705 (common Caucasian subtype) were most predominant in control and SPA groups. HLA-B2702 was found in only one patient with acute anterior uveitis. HLA-B2704 was the predominant subtype in the AS male patients while HLA-B2705 occurred most frequently in females with undifferentiated SPA. ( )

Another explanation of immunogenetic concept of SPA susceptibility (especially suitable for HLA-B27 (-) SPA occurred predominantly among Black Africans and Japanese) was presented by so called “share epitope” hypothesis ( ). Thus, some of residing 8-9% of HLA-B27 (-) SPA patients are carriers of HLA-B7-CREG (cross reactive group) antigens: HLA-B7; Bw-22; Bw-39; Bw-40, Bw-42, Bw-60, -which express or share a common epitope with HLA-B27 and hence could be AS - determinant, rather than HLA-B27. Moreover, AS patients heterozygous for HLA-B27/ HLA-B40 (i. e. shared two haplotypes) could change the AS additive (dominant) inheritance model to recessive, which suggests a synergetic effect operating between this alleles (allelic synergism). However this cannot be the complete picture, since not all of HLA-B27 (-) patients are B7-CREG (+). ( )

As far as the risk of contracting AS among HLA-B27 (+) is relatively low (2-8%) there is of interest to study another non HLA-B27 antigenic associations. Data indicate significantly strong associations between AS and HLA Cw1/Cw2 ( ) and Cw2/Cw6, however, possibly due to high linkage disequilibrium with HLA-B2702 and HLA-B2705 subtypes. ( )

No significant difference between AS patients and controls has been found in either HLA-DR ( ) or HLA-DP loci antigens ( ). However, if assessing the role of other HLA genes not separately, but in association with B and MHC III loci (i. e. evaluating of MHC hyplotype as whole), certain evidence have been revealed. Recently, the high risk genotype for juvenile onset AS was found which consisted of the HLA-B40 (one of the B7-CREG allele shared common epitope with HLA-B27), HLA-DRB108 and HLA-DPB1. 0301 (alleles of MHC II loci - regulators of immune response) as well as the LMP 2 b/b genotype (allele of MHC III locus amenable for encoding of cytoplasmatic proteinase complex which has been applied in processing of foreign proteins into peptides that are subsequently bound by HLA class I molecules, i. e. antigen presentation to CD4 and CD8 lymphocytes). LMP2 b/b genotype was most pronounced among Juvenile AS patients with acute iridocyclitis. ( ) It’s difficult to overestimate the importance of this data because they attempt to disclose whether other HLA encoded genes can further contribute or modulate the end expression of the disease.

Certain strains of bacteria and viruses such as Klebsiella, Chlamidia, Salmonella, Shigella flexneri, Campilobacter, Yersinia enterocolica, HIV and Rubella viruses might be considered as initiating factors of SPA susceptibility.

Evidences concerning the relevance of mentioned above pathogens as initiating risk factors for SPA, are following: 1. Higher than expected occurrence of certain bacterial strains among AS and ReA patients; HIV - among Psoriatic Arthritis and Rubella virus among Juvenile AS patients; 2. There is the strong temporal relationship between Genito-urinary and Gastro-intestinal infection outbreaks and attack rate or cumulative incidence of Reactive Arthritis (ReA) (1-3% for Shigella, Salmonella, Chlamidia and up to 50% for Yersinia) ( ); 3. Discovery of these microbial antigens, but not viable pathogens (excluding HIV - derived SPA) in inflamed synovial tissue ( ) as well as antibodies to the appropriate bacterial epitopes in synovial fluid, sera and also antibodies to **modified HLA-B27 + bacterial antigen** complex fixed in synovial tissue. ( ); 4. Some of these antibodies as well as certain clones of CD4(+) and CD8(+) T - cells specifically directed to bacterial antigens (epitopes), at the same time are cross reactive with HLA-B27 molecules expressed on the surface of synovial cell membranes (phenomenon of molecular mimicry) and hence might trigger autoimmunity process. ( ); 5. Treatment with antibiotics (particularly tetracycline or its derivatives) both shortens the duration of the disease and decreases the rate of recurrent attacks of Chlamidial ReA (but not AS) ( ) provides indirect support of the role of antigen persistence and antigen presentation in the development of certain SPA forms.

The peculiarities of infectious process in SPA were the dissemination and persistency of microbial antigens within host tissues. All of these “SPA causing” pathogens are intracellular inhabitants and are processed intracellularly by the LMP encoded cell proteasome system for further presentation on the cell surface membrane as the antigenic epitope. There is the significant overlap or homology in the certain sequences of the epitopes both derived from different bacterial strains and the hypervariable region of alfa-1 domain of HLA-B27. ( ) Obviously, there are no “exclusively specific” pathogens for SPA, but there are arthritogenic peptides like heat shock proteins of bacterial or viral origin ( ) overlapping with certain subtypes of HLA-B27/B7CREG antigen. The latter is the host receptor for foreign bacterial epitope. SPA susceptibility will mainly depends upon the strength or avidity of HLA-B27/B7 subtypes to bacterial epitope since its presentation and recognition as a whole complex (HLA-B27- bacterial epitope) is the first step of immune response.

Further severity and/or adequacy of immune response developed to the antigenic challenge might be dependent from the “synergism/antagonism” of the combination of alleles allocated in different subloci of MHC (especially MHC class II sublocus). Thus, the

rationale to evaluate as a high risk genotype is not the occurrence of HLA-B27 only, but assessment the combination of HLA antigens encoded by different MHC subloci (hyplotype of the individual with adverse outcomes who carries certain pathogen), i. e. weighing personal hyplotype vs certain pathogen. Such an approach could delineate high risk hyplotype variants regarding the certain type of exposure.

The strong relationship between gut disturbances and SPA bases upon the following events: 1. Almost all of the pathogens presuming amenable for SPA are intestinal inhabitants and arthropathies follow enteropathic infections after few weeks and usually will abrogate six months later. ( ) . Moreover, patients with urogenital (Chlamidial) reactive arthritis may also develop enteric manifestations over natural history of the disease. 2. About 20 per cent of patients with chronic inflammatory bowel diseases (IBD) like Crohn disease and Ulcerative Colitis are associated with or even are the cause of chronicity of peripheral arthritis and AS development. ( ). HLA Bw-62 could predispose B-27/-/ IBD patients to develop chronic arthritis. HLA B-27/B-44 phenotype was found to place patients at high risk of developing both the common manifestations of CD and AS ( ), (so called bowel derived AS) . 3. About 70 per cent of idiopathic AS and more than 80 per cent of patients with undifferentiated SPA have clinically silent ileitis, whose ileocolonoscopic biopsies mimic mild/moderate CD like morphologic pattern. ( ). 4. Sulfasalazine used for years as IBD treated drug is also highly efficient for AS and other SPA forms. ( ). 5. Data obtained from intestinal bypass surgery serve as a human model of SPA . Arthropathy develops in some 20-80 per cent of patients 2-3 months later jejunoileostomy due to bacterial overgrowth and mucosal alterations in the blind loop which probably stipulate a substantial increase of antigen stimulation and further abnormally developing immune response. ( ). Surgical reanastamosis of bypassed intestine is followed by complete resolution of arthritis. 6. Data obtained from transgenic mice or rat models where human HLA B-27 and beta-2 microglobulin genes have been introduced into mice or rat different lines. Spontaneously developed multiorgan inflammatory disease, closely resembled SPA suggests that events initiating disease process occur in gastrointestinal tract. It is of interest that arthritis occurred primarily in male animals and only when mice were transferred from pathogen free barrier facility to the conventional area (i. e. when there was environmental pathogen's exposure. ( ).

All of the arguments presented above point out that primary defect or alteration of intestinal mucosa followed by increased permeability and/or functional disturbances of gut associated (local) immune apparatus might be prerequisites facilitated the contact or invasion of disease initiating factors(pathogen's epitopes) into the host. In other words, gut derived disturbances could be considered as the factors affecting risk of exposure to initiating factors in SPA susceptibility. In sense of pathogenesis, it could be hypothesised

that genetically indetermined or subsequently developed(via previously existed low-grade gut inflammation) increasing of mucosal permeability may overload the local immune apparatus with foreign antigenic material what in turns could diminish the mechanism of “oral tolerance”. This mechanism exists for releasing of antigen material “surplus” from the gut mucosa and operates via Ig A class antibody engagement, that protects the host internal environment from inadequate harmful systemic immune reactions. In the case of absence of the “oral tolerance”, inadequate and not in time suppressed systemic immune response to initiating or arthritogenic patogens could be transformed in autoimmunity owing to existing molecular mimicry between HLA B-27 and foreign antigenic epitopes . ( ).

In conclusion, cosidering SPA causality concept, it must be said, that this group of Rheumatic disorders has multifactorial background where certain environmental initiating factors are known to be related to the development of the disease. However, consistent familial clustering of SPA and immunogenetic marker like HLA -B27 observed in about 90 per cent of SPA patients suggest the independence of such a genetic background from environmental exposure. At the same time, despite the strong association between HLA B-27 positivity and SPA morbidity (RR=100) this relationship actually is too far from absolute. Moreover, HLA B-27 positivity condition is neither necessary (since some 5-8 per cent of AS patients are HLA B-27 negative), nor sufficient (because only 2-8 per cent of HLA B27 positive individuals will contract AS over their lifetime) for SPA . ( ). Meanwhile , epidemiological investigations suggest that probability to contract SPA among HLA B-27/+/ first degree (especially male) relatives of SPA probands could increase up to 25-30 per cent and “true breeding” phenomenon characterizes SPA families. ( ) . It means that HLA B27 positive FDR-s copy predominantly the same SPA entity occurring at their proband. Analogously, Haida and Pima Northern American Indian tribes as well as Inapiat Eskimos both have high prevalence of HLA B-27 (about 50-60 per cent). However, fomer ethnic group is strongly predisposed to contract Ankylosing Spondylitis, while the latter-Reiter`s disease. It assumes that an additional gene/s/ or gene-environmental trigger interaction is required for modifying the final phenotyping expression of SPA. ( )

Probably, Seronegative Spondyloarthropathies might be considered as the best candidates among the Rheumatic disorders for the development of control systems, because not only that they have the precise immunogenetic marker HLA B-27 to identify susceptible individuals, but definitely delineated group of environmental pathogens is implicated as initiating factors, as well as fairly well established factor affecting risk of exposure to such initiating agents is suspected for them . Thus, there is all spectrum of disease causality components, and the challenge to epidemiology is to disentangle this web and measure their relative impact and possible interactions regarding the development of pathological process and final outcome onset.

Based upon the concept of conditional independence, ”gene-environment” interaction might be defined as “a different effect of an environmental exposure on disease risk in persons with different genotypes OR different effect of genotype on disease risk in persons with different environmental exposures” ( ). The existence of interaction will be measured via additive or multiplicative scales suggesting that the effect of exposure on disease risk differs among persons with different genotypes. ($RR_{11} = RR_{01} + RR_{10} - 1$ in additive scale and $RR_{11} = RR_{01} \times RR_{10}$ in multiplicative scale) . It is also important to reveal where the interaction comes from (predominance of attributable fraction of high risk genotype or exposure . Otherwise, to reveal the extent of preventibility.) Recently , five models of relations between a genotype and exposure were suggested by Ottman, 1997. ( ) where different predictions about disease risk in individuals were classified by the presence or absence of high risk genotype(HRG) and exposure (E). Four of them are biologically plausible for testing possible interaction of HRG and E at SPA.

Model # 1 where HRG (HLA B-27 and/or certain HLA -haplotype) could increase the risk to contract SPA via addition or linking to predominated E-effect and have no effect on disease risk (DR) among unexposed : HRG

E ⊥ DR

E

Model # 2 : HRG ⊥ DR In this model E (initiating pathogen) might increase risk to contract SPA among persons with susceptible genotype only and have no effect on persons with low risk genotype.

HRG

Model # 3: E DR In this model the effect of HRG would be restricted to exposed and at the same time E will have no effect on DR among persons without HRG. In other words , there will be no single effect neither HRG, nor E on DR.

Model # 4 : HRG —— DR — E Both HRG and E will each affect SPA susceptibility risk in the absence of other, but the combining effect might fit multiplicative or additive model or neither. Otherwise, there is a single effect both HRG and E on DR. In this model an antagonistic or joint effect less than expected could be revealed.

In first three models interaction would also be antagonistic where HRG, E or both might affect each other and RR11 or SPA risk in that persons could be reduced vs the effect of the same risk factors acting alone.

Thus, there are at least 7-8 possible 'G-E' interaction models for testing data obtained from the persons classified by the presence or absence both of E and G if certain HLA Haplotype will be used as a surrogate of HRG of SPA patients. Regarding further preventive measurements, it`s extremely important to know to what model from presented above the interaction at SPA is obeyed. Understanding of this would help to predict disease rate and provide the basis for well informed recommendations regarding SPA prediction and/or treatment.

ASSESSMENT OF COURSE PREDICTORS IN SPA

Alike other diseases, classification of SPA is primarily based upon the phenotyping expression or clinical signs and symptoms, despite the substantial etiopathgenetical heterogeneity within distinct subtypes of this group. Their Lamping under one "case definition" could result in overlooking the relevant risk factors which might be critical in only one subtype. Epidemiological analysis of such a heterogeneity background of SPA forms could also influence the diagnosis, management and prognosis of individual patient. At the same time, final definition of SPA is also depended upon the stage of natural history. Different forms vary in pattern and speed of progression from the moment of subclinical manifestations to complication onset stage and impairment. Moreover, such a progression might occur among only few affected individuals with certain SPA forms (because of self

limiting course characterizing them), while for others this progression occurs in majority of affecting persons.

Thus, further splitting of SPA both into course stages and certain subtypes can provide insite into pathogenetic mechanisms and factors affecting the clinical overlap and progression speed of disease in individuals. Hence, the knowledge about course predictors ultimately can influence the development of effective prevention and intervention strategies.

Problems that can arise over the approach suggested above are following: 1. Whether the certain susceptibility risk factors (or their combinations) might determine distinct SPA subtypes and have any prognostic value for individual patient to contract the disease. Which of them are amenable for overlapping of clinical manifestations and transformation of one form to an other. 2. Is there any relationship between distinct SPA forms and disease severity or/and final outcome(disability)? 3. What kind of initially or stage revealed clinical and laboratory findings or variables could serve as predictors of poor prognosis in each SPA case observed and what is the extent of their preventability? 4. How could the final outcome be measured and is there any clinical-laboratory variable combination specific for onset of certain type of final outcome? 5. What kind of population at risk is more suitable (in terms of cost-effectiveness) for observation; what sample size, study design and methods of statistical analysis would pertain optimally for resolving the problems aroused ?

As have been said previously, males in general are more predisposed for SPA than females and clinical manifestations of disease is also less severe among women. ( ). However female predominance(4:1) is observed for Psoriatic arthropathy(PsA) and mainly for peripheral arthritic subtype of PsA. For Psoriatic spinal disease there is also male predominance(2:1) ( ). There is no any difference in m:f ratio for undifferentiated SPA maybe due to overrepresentation of cases at the initial stage of disease natural history continuum. ( ). Women Ankylosing Spondylitis (AS) tends to have more peripheral joint involvement and spinal disease progresses less rapidly. ( )

There is an interesting relationship between age of onset of disease, geographic/ethnic background and course severity/adverse outcome subtype. In general, early age of disease onset is more specific for AS where peripheral arthritis pattern is predominated. ( ) However, age of onset as well as involvement of peripheral joints are influenced by geographic/ethnic background. In developing countries of Northern Africa with poor living conditions age of onset less than 15 was established in 25 % of AS patients vs 10% in France. ( ). Moreover, no one from the group of 47 second generation AS “Beurs” born and living in France developed disease before 15( ). Other studies have also shown a low frequency of juvenile onset AS in white population than in people of developing world ( ). Moreover, there is a tendency that AS patients in developed countries have become progressively older at the age to contract the disease. - about 44 in

average. ( ). In turns, early onset of AS is considered as a risk factor of disease severity which leads to early involvement of hip joints and as a sequence to total hip replacement. ( ). The reason of it is that developing child hip may be at greater risk of damage or alternatively more marked inflammatory process may result in a greater joint destruction at JAS patients. Amor 1991 has shown that 30% of JAS patients from Northern Africa had total hip replacement vs 7, 9% in France during the same period of observation. Possible discrepancy in age of AS onset in countries with different socio-economic conditions might be related to a later age of exposure of the presumed environmental trigger or its altered virulence caused by widespread antibiotics` usage. ( ). Family based studies of AS sib-pairs have shown that age of disease onset among them was discordant, while date of onset in each sibling was fairly similar suggesting that trigger exposure was occurred at about the same time. ( )

Hence, preventive measures directed to hypothesised trigger would change the impact of risk factor like " age of AS onset" and consequently the need in hip replacement (outcome).

Data concerning the familial clustering of SPA suggest the occurrence of "true breeding" phenomenon or copy of proband`s disease form in variating clinical extent by the FDR predominantly. ( )

The ability to detect class 1 and 2 HLA-alloantigens as well as their subtypes has allowed not only further elaboration of genetic concept of SPA susceptibility, but also applied for prediction of the development of certain SPA subtypes. Thus, HLA B-27/B40, DR-B1*08/DP-B1*0301 phenotype combining with LMP 2b homozygosity confer high risk to contract JAS and acute anterior uveitis(AAU). ( ). Similarly, HLA B-38 appears to be associated selectively with PsA regardless of the occurrence of its spinal or peripheral subtypes. ( ). Moreover, due to HLA typing there is fairly probability to predict the clinical pattern of SPA (spinal or peripheral), as well as to justify probability of the development of the overlap of some clinical syndromes. SPA patients (regardless of occurrence definite form) carried HLA B-27 are more proned for sacroiliitis(SI) , spondylitis(S) and AAU, whereas peripheral arthritis is often associated with HLA-DR4. ( ). Likewise, combination of HLA B-27 and Bw-62 is more specific for IBD-derived AS. ( ).

Given high risk HLA genotypes determine only possible increased susceptibility to SPA, however, final phenotyping expression is ultimately depended upon "'G-E"' interactions and other unrevealed causes.

The importance of specific environmental pathogen regarding severity of clinical expression could be demonstrated considering HIV-derived SPA. In setting of HIV infection these entities are often distressingly severe illnesses where the hallmarks between them are blurred and the development and progression of clinical manifestations(mainly systemic)

become fulminate and unconstrained at the given immunogenetic background similar to HIV negative SPA persons. ( ). Curiously, classic AS is not seen among HIV-derived SPA maybe due to fulminate course of HIV infection, not enough for developing of typical findings . ( ).

Examples elucidating the substantial role of factors affecting risk of exposure of suspected arthritogenic pathogens over clinical manifestations of SPA are reactive urogenital and enteropathic arthritides (ReA), as well as arthropathies following chronic inflammatory bowel diseases(IBD). There is the strong temporal relationship between acute local gut and urethra inflammation and the onset of arthritic syndrome. ( ). Reciprocally, the chronic course or inadequate treatment of urethroprostatitis/cervicitis and enteropathy leads to chronicity, impairment and functional loss of locomotor apparatus. ( ). Enteric exacerbations in IBD patients are followed by the attacks of peripheral arthritis. ( ). Recent studies seems to indicate that treatment of nongonococcal urethritis by tetracycline ( ) and IBD by sulfasalazine significantly reduces frequency of arthritic relapses. ( ). Moreover, number of patients with sexually acquired ReA observed in recent years has notably decreased probably due to in time diagnosis and adequately treatment provided. ( ). Considering that about 20 per cent of them could be candidates for chronicity and disabling SPA forms, it's possible to appreciate that the last 2 groups of risk factors are targeted for preventive measurements implementation.

The next step providing the background information for rational preventive and curative strategy might be the tracing persons with HRG and/or exposed along the natural history continuum(from subclinical stage of disease to final outcome) aimed to reveal possible process variables indicative for poor prognosis in SPA patients.

In contrast to other Rheumatic disorders, in SPA the primary pathological feature is the inflammatory changings allocated at the sites of ligamental or capsular insertion into bone (so called enthesopathy) and characterized by fibrosis and ossification rather than joint destruction and instability. ( ). The enthesis(sites of insertion) are metabolically active sites of locomotor system, perhaps explaining why early changings occur during growth in teenage years. However, there are also peripheral joint involvement in SPA , as well as involvement of extraarticular organ/systems over course of disease. Thus, generally, SPA combine four main syndromes: enthesopathy, pelvi-axial or spine, peripheral arthritic and systemic or extralocomotor. Their combination varies from one SPA form to other, patient to patient, and in a given patient, during the course of disease. The question is, in what extent the occurrence of any clinical feature within these syndromes or their combination might have prognostic value; whether the clinical picture at onset of disease at given susceptibility factors can predict further clinical course and outcome in any individual patient?

In childhood the onset of SPA is peripheral arthritic predominantly and presented by so called seronegative enthesopathy/arthropathy syndrome (SEA): enthesopathy, asymmetric mono-, pauci-, polyarthritis predominantly of low limbs. ( ). Although about 80 % of SEA patients develop further any definite SPA entity (predominantly AS), this atypical or undifferentiated subtype is difficult to differentiate from juvenile onset rheumatoid arthritis until the development of the classical signs of pelvi-axial syndrome over several years during the course of disease. ( ). However , several signs like male gender, >10 year age at onset, familial history of any SPA form, onset of arthritis following an idiopathic enteritis, presence of HLA B-27, as well as negativity for rheumatoid and antinuclear factors, in 80-85 % of given SEA patient might help to predict possible SPA with sensitivity 85% and specificity 100%. ( ).

As a predictor of poor prognosis might serve also polyartiarticular (more than 5 joints) pattern of onset, especially if followed by systemic manifestations like high grade fever, skin, nail, mucosa involvement (but not ocular involvement). This is well demonstrated in the setting of HIV positive patients. ( )

Another bad course predictors are duration of clinical signs and symptoms more than 6 months from the onset (major sign of transition to chronicity of in inflammatory process); frequency of recurrent arthritic and enthesopathic attacks, as well as systemic flaws ( ).

The rapidity of the development of complication stage of SPA like skin/mucosa, cardiac and pulmonary involvement followed by functional impairment as well as the speed of progression also could be considered as poor prognosis features ( ).

From the laboratory findings steady elevated ERS and other acute stage inflammatory estimates, hypergamma -, alfa2 -globulinemia, anemia and serologic evidence of the persistence of certain pathogens over natural history are also determined as poor prognostic measures( ).

The early presence of peripheral arthritis, iritis, pulmonary fibrosis and persistently high ERS rate is also indicative of poor prognosis ( ). Long term disability and sick leave are higher in SPA patients with work exposure to cold conditions and prolonged standing ( ).

The occurrence of certain antibodies to environmental pathogens cross-reactive to HLA B-27 as well as quantitative and functional disturbances in T-cell immunity are also amenable for undesired outcome ( ).

The poor prognostic factor is the decreased efficiency of routine treatment regimen such as NSAIDs at the certain SPA patient ( ).

The other serious problem needed to be solve, is how to measure outcome of the disease. The lack of systematic approach to SPA assessment is the primary disadvantage of the majority of follow up studies. The systematic approach implies to encompass all of the

manifestations revealed over natural history of disease, i. e from subclinical stage (HRG, environmental E) through the preclinical stage (clinical features at the onset) and complication stage (clinical features of definite SPA entities, their severity as intermediate process markers of the disease) to final outcome (disability, handicap, and death) as the temporal continuum where there might be enormous risk factors dynamically interacted and determining further clinical manifestations. Hence, the measurement of outcome is the dynamic process which includes the assessment of impairment (anatomical defects of organ/systems caused by disease, i. e manifestations of the SPA complication stage), disability or functional loss of organ/systems caused by impairment, i. e functional disturbances at advanced SPA cases and handicap or social disadvantage produced by the disability ( ).

It is obviously that study design pertaining the purposes suggested above could be long term prospective follow up or cohort of healthy HLA B-27 positive first degree relatives (FDR) and/or with yet undifferentiated SPA who might be traced continuously until the onset of final outcome. The rational of the option like this is based upon the assumption that the prevalence of SPA (predominantly undifferentiated forms) among these FDRs is more than 20-30 times higher than in general population and the frequency of HLA B-27 genotype attains up to 50 per cent among them vs 6-14 % in Caucasians. Hence, 1 from 3-4 FDRs of SPA proband is proned to develop SPA over life time ( ).

CURRENT SITUATION OF SPA MORBIDITY IN ARMENIA

Unfortunately current comprehensive data concerning the epidemiology of SPA morbidity in Armenia are not available because lack of any explorations performed directly in this area till now. However, certain image about this problem might come from the information based upon the analysis of hospital records of patients with different Rheumatic Diseases treated in the department of rheumatology of "EREBOUNY" Medical Center over 11 years (1986-1996).

The main causes of 6144 hospitalizations were degenerative joint disorders (osteoarthritis and spondylosis) - 51 per cent of all hospitalisations; Rheumatoid Arthritis -16 per cent; Seronegative Spondyloarthropathies -12 per cent; miscellaneous group of Soft Tissue Rheumatism (tendinitis, bursitis, capsulitis, myofascial syndrome and fibromyalgia)-6 per cent;microcristalic arthritis (gout, psudogout) -4 per cent and Diffuse Connective Tissue Diseases and Systemic Vasculitis (SLE, SS, DM, MCTD and NPA) -2 per cent and other - 9 per cent.

371 patients with different SPA entities were revealed over 11 year period. The profile of them was as follows: Idiopathic Ankylosing Spondylitis-102 or 27, 5% from 371 SPA; Ankylosing Spondylitis due to (or coexisting with) Inflammatory Bowel Diseases- 14 or 3, 8%; Reiter`s Syndrome- 108 or 29,1%; Enteropathic Reactive Arthritis and or Undifferentiated

SPA - 69 or 18, 6%; Psoriatic Arthritis -53 or 14, 3%; Behcet`s Syndrome- 18 or 4, 85% and SPA transformed from Juvenile Chronic Seronegative Pauci Arthritis- 18 or 4, 85%. Final diagnoses were revised due to retrospectively reassessment of clinical manifestations of SPA patients` hospital records in accordance with currently existing classification criteria both for SPA as whole as well as for definite SPA entities. ( ). 18 patients with pauciarticular peripheral arthritis and enthesitis with age of onset less than 16 were observed in our department(for adult rheumatic patients) after 3-5 years and their clinical features did not meet classification criteria for any definite SPA forms. Eight of these 18 patients had also unilateral grade 2-3 sacroiliitis, 12-only seronegative peripheral arthritis of low extremities followed a short episode of acute enteritis at the onset of disease. There were no any inflammatory enteritic flaws in future. All of them were ANF(-) and male:female ratio was near equal to 3:1. Skin manifestations like psoriasiform eruptions were occur in 4 sacroiliitis free patients and mild/moderate conjunctivitis occurred in 4 patients. However, further follow up observations were not available.

Clinical comparison of IAS, RS, ReA and PsA with axial(not peripheral) involvement showed male predominance(from 6:1 for IAS to 3:1 for PsA). Early age of onset(less than 30) was established only for IAS patients. The initial articular manifestations occurred usually in younger age group regardless the certain SPA forms. Initial peripheral arthritis was found in IAS(46, 2%), RS/ReA (84%) and PsA(90, 3%). For latter group distal interphalangeal joint involvement occurred in 38%. Low back pain as the main complaint was found in IAS (86), RS/ReA (32%), and PsA (14, 8%). Radiologically sacroiliitis was found in IAS (100%), AS due to IBD (92%, in 46% bilateral, but not of similar extent), RS/ReA(38%-predominantly unilateral) and PsA(44, 8% unilateral and predominantly in males- 4:1). Classic features of Spondylitic disease was found predominantly in patients with IAS(48, 6%)and AS via IBD(27, 6% and there was the skipping of radiological features over lumbar portion of spine.). Other associated symptoms were similar for comparing groups. Particularly, evidence of enthesitis, polyarticular pattern were less common than monoarticular involvement. Hip joint was involved more commonly in patients with IAS and associated especially with early age of disease onset. Among all IAS and IBD-AS six patients had uni-4 and bilateral-2 hip replacement. There was no any patient with atlanto-occipital subluxation or involvement of temporomandibular joint.

Behcet`s Syndrome occurred in our clinical material with sunstantial frequency and manifested via peripheral mono/olygoarthritis predominantly allocated in low limbs in 14 patients. Sacroiliitis was revealed only in two patients. Nobody of them had Spondylitis. The main extaarticular manifestation was uni/bilateral posterior uveitis associated with blindness in 4 patients(from 8 patients with uveitis). Positive pathergic test of skin as well as erythema nodosum were observed at 16 patients.

More than half of the patients with ReA following acute enteropathy (39) were serologically positive for Yersinia Enterocolica, and erythema nodosum was also observed at 21 from these 39 patients.

Although the presenting data reflect the profile of SPA entities and have little epidemiological significance and not generalizible, however, they suggest that SPA problem actually exists among Armenian population(maybe with not less prevalence rather than Rheumatoid Arthritis) and has substantial peculiarities regarding BS, Yersinia derived ReA and frequently involvement of hip in young AS patients.

The another source of information related to Rheumatic diseases is "Annual Morbidity Record Form" preparing by Statistical Department Ministry of Health. Unfortunately, in this record form SPA morbidity is not presented in separate issue, but combined with other inflammatory joint diseases like Rheumatoid Arthritis, Gout as well as Diffuse Connective Tissue Diseases (DCTD). In other issue of this review overall morbidity of Muscle-Skeletal (M-S) and Connective Tissue Diseases were also presented (including injuries and genetic disorders). Tables 2 and 3 show both overall morbidity trends (prevalence and incidence) of "Rheumatic Diseases" and Inflammatory Joint diseases with DCTD among child (< 14) and adult population in Yerevan and Armenia over 1990-1996. There are severe decrease of prevalence of overall M-S disorders amongst adult and child population both for Yerevan and Armenia and less severe decline of cumulative (RA-SPA-DCTD) prevalence amongst the same population. Cumulative prevalence of Chronic Inflammatory Joint Diseases and DCTD in Armenia and Yerevan is significantly higher amongst adult rather than child population.

However, presenting data are not reliable, since there is no any significant decrease in “incidence rates” of similar groups of interest over the same observational period. This bias might occur due to low attendance of chronically ill patients to the polyclinic rheumatologist, but not the patients with newly revealed disease, who might be in acute stage and suffered mach more intensivelly.

Another reasons of bias could be the artificially combining the diseases with different background into the same issue in statistical report as well as the absence of the age and gender adjustments of presenting morbidity data.

There are also data concerning the distribution of HLA -A and B subloci alloantigen phenotypes among 58 AS and 50 ReA Armenian patients. The same groups of patients were also tested over 29 phenotypes of 7 erythrocyte isoantigen system(ABO, Rh/Hr, MN/Ss; Pp; Kell- Chellano; Duffy; Kidd). Diagnosis of AS and ReA were established according to Rome diagnostic criteria for AS. (Kellgren 1963) and Calin et al criteria for ReA (1991). The testing of erythrocyte system was performed by hemagglutinetion and indirect Coombs test, and HLA typing due to microlymphcytoxic Terasaki technique using

monospecific antysera (Biotest-Serum) against HLA-A and HLA-B antigens. Control group included 1100-48892 healthy blood Armenian donors (sample size variation was depended on the system tested).

It was ascertained that both positive and negative associations of AS and ReA patients were related with the following phenotypes: positive AS and ReA associations with HLA-B27 and SS; negative - with HLA-A11; HLA-B12; HLA-B13. Relative risk to have AS for HLA-B27 carriers was equal 73, 33 and to have ReA for HLA-B27 carriers was equal 10, 2. The associations of moderate degree were also observed between AS and HLA-B35 (RR=3,09) and SS (RR=2, 11) and between ReA and SS erythrocyte antigen (RR=3, 4) (Nersissyan et al, 1994).

Armenian population is fairly well characterized regarding the prevalence of antigen systems presented above. These data are based upon the representative sample sizes varied from 1100-48892 blood donors. The prevalence of HLA-B27 antigen among Armenians is equal to 6 per cent. The frequencies of HLA-A sublocus alleles are distributed as follow: A2 (0, 24)>A3 (0, 17)>A9 (0, 13)> A1 (0, 116)> A10 (0, 109)> A11 (0, 095)>A28(0, 03). For HLA-B sublocus alleles their frequencies are: HLA-B7 (0, 185)> B12 (0, 182)> B5 (0, 17)> B8 (0, 139) > B13 (0, 06) > B18 (0, 03) > B17(0, 027) > B35 (0, 036) > B27 (0, 03) > B40 (0, 02) > B16 (0, 018) > B14(0, 016) > Bw21 (0, 0016) > Bw22(0, 012) >B15 (0, 0115).

Most frequently occurred phenotypes for HLA-A sublocus are A2/A3; A2/A9; A1/A2 and A3/A9. For HLA-B: B7/B12: B5/B12: B5/B7; B8/B12.

Most frequently occurred haplotypes are: A2-B12; A2-B5: A3-B7; A1-B8.

The frequency of HLA-A28 and HLA-B27 alleles among Armenians are small, however, the occurrence of HLA-A28 - HLA-B27 haplotype is fairly high (0, 01). Haplotypes like A3-B13; A11-B27; A28-B8 are absent in Armenian population. Among the same population there is evidently strong gamet linkage for A3-B12; A2-B12; A3-B7; A2-B5; A1-B8 HLA-A-B subloci alleles.

It is of interest that frequency of HLA - B27 antigen is 6 per cent, while HLA-B7CREG superantigen is occurred most frequently in Armenians tested for HLA (33, 5% of 1481 blood donors).

There is a high clustering of erythrocyte system for genetic markers: K (0, 95); Lu^b (0, 94); e (0, 82); D(0, 65); s(0, 62); Pa (0, 35) - high clustering. Moderate clustering for Io (0, 53); Le (0, 56); M (0, 57); N (0, 42); Fy^b (0, 57); Fy^a (0, 43); Ik^a (0, 52); Ik^b (0, 48); P_1 (0, 53); P_2 (0, 48); c (0, 54); C (0, 45); Le (0, 44). Low clustering: K (0, 04); Lu^a (0, 06); q_b (0, 11); E (0, 17); d (0, 35); S(0, 37). Di^a - gene is absent in Armenian population.

The occurrence of Ig heavy chain Gm- genes are as follow: Gm^b > Gm^a> Gm^{ax} (0, 67>0, 23>0, 001 accordingly). The distribution of their phenotypes in Armenian population are: Gm (a- b+ x-)> Gm (a+ b+ x-) > Gm (a+ b+ x+)> Gm (a+ b- x+)> Gm (a+ b- x-).

It is also of interest that there is strong relationship between HLA-A3 - B7 haplotype and Fy (a- b+) phenotype and Multiple Sclerosis morbidity among Armenian population. ( ).

The comparative analysis of genetic and immunogenetic marker distribution convincingly argues the proximity of Armenians to Caucasians rather than to other ethnicities. Hence, the expected SPA morbidity among Armenians (in given exposure situation) would compatible with the analogues indices among Europeans.

In conclusion, it's necessary to emphasise that SPA morbidity is also the relevant Public Health problem for Armenia, although there is no comprehensive epidemiological data. The existing rheumatologic facilities allocated in more than 50 polyclinics(child and adult) and 3(two adult and one child) hospital based Rheumatology departments in Yerevan (population size is equal about 1200000 and population homogeneity as to be Armenian - up to 93 per cent) could make feasible to carry out an exploration concerning the assessment of susceptibility risk factors and course predictors of seronegative spondyloarthropathies. There are also appropriate scientific-research institutes in Yerevan, could be able to perform the necessary investigations.

The basis or first step of proposed research should be the reorganisation of the structure and activities of existing city rheumatological service alike "Arthritis Surveillance System" aimed to acquire data on prevalence of Rheumatic Disorders(particularly SPA), seeking the risk factors of subsequent disability via community based surveys and finally to control them by prevention and treatment (at the onset) of their complications and disability.

The reorganisation procedure seems not to be much resource consuming since assumes to involve mainly the existing staff needed only for additional training in accordance with newly accepted purposes. Type of this system should be total including all of the rheumatologic facilities mentioned above, which might operate both passively(due to patient's attendance)as well as actively(due to periodically conducting community based surveys). Thus, ongoing systematic collection of data of interest will be attained by the rheumatologists at the primary study sites-rheumatological cabinets of city polyclinics

MANAGEMENT STRUCTURE OF THE "ARTHRITIS REGISTRY" SURVEILLANCE SYSTEM

It's implied that the system will have three levels. The first or lowest level will have to be the primary study sites or rheumatological cabinets in each outpatient clinics. The staff will consist of the rheumatologist (MD), authorised for examination, treatment and implementation of preventive measures of patients over follow up, and nurse of

rheumatalogical cabinet will be served also as data clerk. The duties of data clerk will include correctly completion of data collection forms, maintain study files (under the supervision of the physician); maintain tickler system so that patients late for the scheduled shot might be identified; make home visits (contact to patients do not return for follow up); respond to queries from the study site coordinator and point out to him problems in data collection, follow up or related activities. The nurse of rheumatological cabinet will also participate in periodically conducting community based surveys as an interviewer.

Reports from the primary study sites will be presented to the next structure level of surveillance system weekly.

As the second facility level — the hospital based rheumatological departments will have to be used and supervise, coordinate the activities of the primary study sites (each of adult department-25 polyclinics of its region and child department-all of the child polyclinics). These departments would also be responsible for additional examination and treatment of severe diseased patients with high risk to develop adverse complications and early disability onset. It would be encouraged the temporal transition of MD and nurse staff between outpatient clinics and departments periodically. The department MD staff as well as some department nurses will participate in conducted periodically community based surveys.

The Heads of the departments will have to be the coordinators of second level study sites whose duties will include monthly visits and consultations in each primary study sites to check up the study complience, seek responses to quires from the data entry unit on Coordinating Group, solve problems which may arise, supervise study staff at the primary clinics and hire/fire staff if necessary; coordinate the study files transfer within primary clinics and department to be ensure their maintenance; respond to queries from Coordinating Group and if necessary point out possible changes in data collection forms and process which would reduce the errors made; to evaluate periodically a random number of case reports regarding their compliance to study protocol (reliability and validity of the data collected). The coordinator of second study level in cooperation with all of the rheumatologists of the region supervised by himself is obliged to perform primary analysis and classify the accumulating data of interest in accordance to study protocol data analysis forms and to prepare comprehensive reports for presenting to Coordinating Group. The regional study coordinator, at the same time is a executive, permanent member of Study Coordinating Group. Operational group existed at the regional coordinator will consist of the 3-4 rheumatologists of the department and/or rheumatologists of primary study sites in accordance with regional coordinator decision/will. Apart from MD, Field director (nurse) might also be the permanent member of operational group and coordinate daily activities of data clerks at primary study sites and at the same time would be the data maintaining clerk

of the department. The data collection forms should be reported to the Study Coordinating Group monthly.

The role of Coordinating Group (third level) is to create study protocols and study design concerning the different topics of certain Rheumatic morbidity of interest; to prepare pertinent record forms; planning implementation of new programs and coordinate the day-to-day activities of current studies; processing data collected from the second level study sites; undertaking monthly quality assurance checks; analysing the data in accordance with the data analysis plan; dissemination the current and final results as widely as possible (to primary and secondary study sites as feed-back information and operational guidelines; to Municipal Health Department and Curative-Preventive Department Ministry of Health as recommendations for improvement and planning Rheumatologic Service activities and needs; to Medical Expert Committee of Ministry of Welfare as a background for creating Disability and Handicap Criteria assessing patients with Rheumatologic Disorders; publications of major study findings in mainstream journals etc.).

In general, the Study Coordinating Group is responsible for data accumulation and analysis; evaluation of the process activity of the surveillance system as well as the dissemination of the current and final results.

The Chairman of Study Coordinating Group should be the Chief City Rheumatologist experienced also in area of the Epidemiology of Chronic Diseases.

Apart from the Second Level Study Site Coordinators, the Study Coordinating Group will include senior epidemiologist familiar with Genetic and Chronic Disease Epidemiology; consultant -statistician; programmer; statistical assistant and secretary. As a invited temporal members involved for creation and implementation of certain programs Rheumatologists and General Practitioners from the Primary and Second Study Centers might also be included.

As a first trial for probing the functional capacities of the Arthritic Surveillance System, we intend to conduct the study concerning the prevalence of Undifferentiated SPA forms among the first degree relatives of SPA-probands; to assess possible risk factors of SPA susceptibility and to reveal clinical predictors of poor prognosis over natural history of the SPA entities.

The rational to use FDRs as population of interest is based upon the empirical observation that SPA prevalence (relatively rare disease group among general population about one per cent) has familial clustering tendency more than 20 per cent and familial accumulation of HLA-B27, (mmunogenetic marker highly specific for SPA patients) about 50 per cent (vs 6-14 per cent among healthy Caucasians).

It is reasonable to study FDRs with Undifferentiated SPA forms, since there is an opportunity to seek for risk factors and transitional pathways of these "harvested" population

to definite SPA entity onset and further disability development over possibly abridged disease course. It might permit to use much less sample size and to shorten study follow up period.

Finally, usage the option of ongoing prospective cohort as a study design could allow to investigate purely the dynamics of "Cause-Outcome" relationship over disease developing process, as well as to evaluate possible risk factors, "Gene-Environmental Exposure" interactions and their impact over natural history.

METHODOLOGY

Introduction. The definition of the problem is the reference point for study planning process which will determine the main Research Question(RQ), Objectives(O), and Hypothesis(H) of undertaken exploration. Consequently all of these statements will dictate or guide the peculiarities of further steps of study design(defining variables to be measured; planning the analysis of data obtained; choosing the approach for actually collecting data; defining sample size and drawing the sample; creating the list or protocol of examination activities for diagnosis and natural history monitoring; collecting, processing the data of interest and preparing them for final analysis and statistical inferences).

The Topic of Interest of the study concerns the SPA causality and prognostic value of certain genetic, environmental and clinical findings revealed at the patients with Undifferentiated SPA in families where there is at least one patient with any advanced(definite) SPA entity or Index Case(IC) .

The main RQ. Is the incidence of SPA derived Adverse Outcomes(AO) among the "Exposed" (with High Risk Genotype and/or exposed by the certain strains of pathogens) FDRs with yet undifferentiated SPA greater and established earlier rather than the " Unexposed" ones (in families of SPA probands living in Yerevan) who will be traced over long-term(1998-2008) prospective follow up?

What Kind and How could these possible Genetic and Environmental Risk factors provide poor prognosis?

Is there any of them preventable or curable?

The main O is to explain whether the incidence rates of SPA derived AO are related to "Exposure" Differences occurred among the FDRs with yet Undifferentiated SPA or to test the extent of impact of the risk factors and their possible interaction concerning the development of adverse outcomes over natural history of the disease.

Study Hypotheses to be tested : 1. Incidence rates of certain AO would be higher among the "Exposed" FDRs with yet undifferentiated SPA vs " Unexposed". 2. There will be "Gene - Environmental Exposure" interaction regarding the development of certain AO over

natural history of the disease. 3. There are more likely to occur certain preventable or curable variables amongst the risk factors considered.

Thus, the statements presented above point out the type of study design(prospective cohort where the impact of risk factors would be measured directly and dynamically), target population (FDRs of Index Cases who have contracted yet Undifferentiated SPA), systemic survey approach to SPA families for multistage sampling and data collection(list of all Index Cases already revealed via hospital records-survey amongst FDRs of IC aimed to reveal patients of interest-their examination and taking eligible ones under follow up), defining Independent Variables (imunogenetic, immunologic, environmental pathogens and variables affecting of exposure of initiating factors, clinical variables revealed at the onset of the disease) and Dependent Variables(incidence rate of certain adverse outcomes) to be measured.

Hence, the main characteristics of study proposed is not to describe the prevalence and family clustering of SPA and spectrum of risk factors observed, but to detect the peculiarities of natural history process viewed from the point of "Exposure"(G-E) impact on it.

STUDY DESIGN

A long-term perspective follow up study of the natural history of undifferentiated SPA cohort is necessary for the evaluation of the effect of certain risk factors (and/or their combination) on the development of the adverse outcomes (i.e. evaluation of prognosis in each individual case). It is important to assess longitudinally all spectrum of SPA adverse outcomes due to direct calculations of their incidence rates as well as to measure the speed of changes in certain clinical signs and symtoms of interest over the disease course (i.e. progression rate).

This type of study design is also well suited for prospective assessment of " Exposure" and covariates.

Planning to use different diagnostic criteria for SPA patients ascertainment (more sensitive for revealing undifferentiated SPA at initial stage of study and more specific for sustainind SPA entity developed) it could be possible to minimize information bias concerns to nondifferentiate misclassification. Selection bias might be controlled at the stage of study design since the proposed study is population based survey encompassed almost all types of SPA families and precisely describes all of selection process as well as at the implementation and data collection stage (selection bias via attrition). Certain group of "process variables" like age, gender, geographical relocation, "return to work ", "loss of interest", activities of daily living (ADL) scores, SPA severity scale, weakness scale, accessibility of HC facilities would be used for analysing the attritional causes over follow

up. Another way for diminishing attritional impact would be the substatially large sample size of SPA patients under the follow up, as well as optimally operating surveillance system.

There will be clearly difined scheduels for annually check up for patients under follow up and the monitoring the appropriate shots by the computerized tracking system.

The staff of primary study sites will be authorized to make home visits and to contact with patients (or their relatives) who do not return for follow up (or to identify the reasons of case loss).

As mentioned above, the survey among "harvested" population will be contracted for screening eligible research units for follow up, that permit to obtain sufficient size (400-450) of cohort with this relatively rare diseases.

An other weakness of this study design - Cost - is not much important for Armenia considering low regional salaries for medical staff (MD about $ 30-40 and for nurses $ 15-20 per month) and owing to already existing rheumatological care facility set supported by the state budget. An additional wage about $ 10- 30 per person-month would be enough for implementation and maintaining the proposed project. Time period necessary for follow up study providing will be equal 10 years (2-3 years for recruitment and 5-8 years for follow up).

However, another comment concerning study design argues that when epidemiological concepts are applied to the analysis of family aggregatrion data, an overlap between case-control and prospective cohort is seen. If positive family history is considered as an exposure factor of cases and controls themselfs (i.e. having affected relatives) the odds ratio represents the ratiio of odds to be exposed in cases vs controls. In cohort study design the exposure is being the relative of the cases or controls and the association measure is the ratio of disease frequency in SPA families vs disease frequecy in non-SPA families. When odds of disease are considered as a measure of association, then both case-control and cohort approaches yield identical estimates of the odds ratio.(.....)

POPULATION TO BE STUDIED

SAMPLING DESIGH

Two methods of family aggregation study : abbreviate family history approach and family study approach, will be used for survey conducted where the sourses of information would be patients with definite SPA (index cases) and FDRs (parents, offspring, sibs) of index cases.

The **Target Population or study universe** will be families where there is at least 1 patient with any definite SPA - index case registried at any of the HC facilities of Yerevan. The comprehencive and more precise list of SPA patients living or lived in Yerevan will be the frame for abbreviate family history survey aimed to reveal FDRs with SPA. As the

survey instrument the special questionnaire would be used asking about the presence of the disease in the family as well as including items about precise diagnosis of index case and s/his demographic data. If it would be impossible to contact with index case (who might be deceased, absent or child), family members could be interviewed for the same purposes. The main screening question would be whether there is any of FDRs have the same or similar complaints. If the answer is affirmative the family would included in the study, if not - dropped out in general. This is a screening type of probability sample design for sampling "rare" population. However, attempting to control selection bias some proportion of those who said "*No*" to the screening question should be included in the sample anyway to permit the rate of false negatives to be estimated among those who were screened out of the study.

As a result of the survey the **sampling frame** of interest (FDRs of actually proved SPA families) would be gained. At this stage of sampling design the family study approach survey would be implemented using questionnaire for detailed inquiery and evaluation of FDRs in terms of eligibility for further physical examination. Personal interviews with FDRs would be provided by primary study site data clerks aimed to obtain sufficient response rate (70-80%). FDRs with 50 per cent probability to have SPA (based upon the survey questionnaire scoring) would be taken for detailed physical examination and only those FDRs fulfilled the diagnostic criteria for undifferetiated SPA for adults (Amor, 1990) and for children (Hussein, 1989) would be eligible for further long term follow up study as **sampling elements** or **sampling units** of the investigation.

Also suggested diagnostic criteria have sensitivity - 91, 9% and 84, 6% and specificity 97, 9% and 100% for adults and children respectively, however, to control selection bias, some proprtion of FDRs not completely meet diagnostic criteria also would be included in follow up as a separate group.

Another eligibility criteria for FDRs is the onset of disease at the age between 10 and 45. Patients less than 10 years old at the onset could not be eligible due to high probability of juvenile onset Rheumatoid Arthritis (especially for girls). Patient more than 45 will not be eligible due to increased probability to have degenerative spine and joint disease which is poorly differentiated from SPA..

The exclusion criteria will be also: the presence at the patients antinuclear antibobies and rheumatoid factor as well as clinical manifestations similar with the criteria for SAPHO (synovites, acne, palmoplantar pustulosis, hyperostosis, aseptic osteomyelitis) syndrome (Chamot 1987) and Familial Mediterranean Fever (Reiman, 1976); Juvenile Rheumatoid Arthritis; Degenerative Spine and Joint diseases (osteoarthritis and spondylosis).

Another set of diagnostic criteria specific for AS, PSA, ReA/RS, IBD derived SPA and juvinile onset SPA will be used over the follow up to identify the onset or development of definite SPA entities during the natural history of undifferentiate SPA paients.

CALCULATION OF SAMPLE SIZE

Several assumptions will be needed for calculation of sample size when conducting family history study survey. If prevalence of disease is identified as a major study variable it must be assumed that: 1) in SPA families this measure more than 20 times higher at HLA-B27 positive FDRs; 2) about 50% of these FDRs are HLA-B27 carriers; 3) the probability to contract SPA over their life time among HLA-B27 positive FDRs is equal 25-30%; 4) the prevalence of HLA-B27 among SPA patients is about 90% in average.

The formula for calculation initial sample size is the formula suggested the standard error at 95% confidence. Hence, standart error would be equal 5%.

n=1, 96^2[P*(1- P)]:p^2=3, 841(0, 2*0, 8):0, 0025=246.

This estimate needs for following adjusments in obtaining the actual or real sample size: 1. for estimated sample design effect(DEFF=1, 3). 246 * 1, 3= 320.

2. adjusment for expected Response Rate of survey(RR= 80% or 0, 8). 320:0,8=399.

3. adjusments for eligibility as HLA B-27 carriers (what is 50 % probability). 399:0, 5 = 799 and adjusment for probability to contract SPA for HLA B-27/+/ FDRs(what is equal 30% or 0, 3). 799:0, 3 = 2663.

Thus, about 2700 FDRs of index cases with different definite SPA entities will be surveyed or screened for revealing about 400-450 patients with undifferentiated SPA who could be eligible for further long-term follow up study.

It is estimated that this final sample will provide 80% power to investigate possible course predictors and will give sufficient number for 5-8 year follow up even if expected attrition rate would be equal to 10%.

IMPLEMENTATION

INTRODUCTION

Undifferentiated SPA encompasses atypical forms of seronegative sponyiloarthropathies (predominantly among HLA -B27 positive FDRs of SPA probands) whose clinical manifestations expected to be similar to proband's disease but yet would not satisfy any of diagnostic criteria established for certain definite types of SPA. Examples are: spondylitis/sacroiliitis without radiologically justified erosive disease; incomplete forms of Reiter's symdrome; juvenile onset seronegative poly/olygoarthritis and/or enthesitis (SEA) syndrome; isolated dactilitis ("sausage" digit), heel pain due to calcaneal/tarsal periostitis. ( )

Thus, they could be cosidered as SPA cases in early stage (yet not uncovered completely) or slowly progressing (due to unknown reasons) SPA forms.

Hence, investigation of the natural history of these patients aim to reveal possible risk factors and threir influence is to a large extend targeted toward primary secondary and tertiary prevention strategies of the disease.

The activities would be directed to identify FDRs with current health conditions (high risk genotype and/or certain types of local inflammatory process of genitourinary system or gut) likely to jeopardize the adverse immediate and intermediate outcomes or identifying FDRs with definite SPA entities already developed and likely to jeopardize the final adverse outcomes (certain types of diability etc.)

Attempts regarding the early detection and treatment of these conditions or at least sensitizuing SPA patients and their families to health conditions likely to jeopardize all of the adverse outcomes of SPA ensuring an appropriate response from them.

As the **INDEPENDENT VARIABLES** to be screened for revealing possible risk factors the following data derived from the survey and initial or entry physical examination of FDRs with yet undifferentiated SPA will be used:

1. Demographic characteristics and living conditions such as age, gender, age of onset or duration of the disease, marrital status/family size, type of SPA bred true in family, work status/nature of work, occupation changing via the disease; income, education, addictions.

2. Genetic and Immunogenetic characteristics: HLA-A, B, C, DR/DQ/DP antigen typing ; Erythrosyte Isoantigen System (ABO, Rh/Hr, MNSs, Pp, Kell-Chellano, Duffy, Kidd) typing with further calculations of their allele, genotype, haplotype and phenotype frequencies.

3. Environmental pathogen exposure characteristics: occurence and sera titers of antibodies against Chlamidia trachomatis, Yersinia enterocolica, Shigella flexneri, Campilobacter jejunei, Klebsiella and certain strains of Salmonella, as well as antibodies cross-reactive with HLA-B27.

Antinuclear antibodies and Rheumatoid factors should be determined for exclusion criteria purposes.

4. Clinical and insrumental/laboratory characteristics at the entry into follow up or during first 12 month of disease onset: pattern of clinical signs and symptoms(see bellow) over first year of the disease onset; acute phase inflammatory indeces(ESR, WBCC, C-RP, Ig and complement lenel in sera), routine blood biochemical measures(Hb, glucose, biliroubin, Cholesterol, BUN and Createnine, Uric acid, gamma GTP, CPK), urine analysis(protein, RBC, WBC, casts); bateriological faecal analysis for gut flora and disbacteriosis;instrumental diagnostic tools (radiography of spine, peripheral joints or chest if clinical indications occurred; ECG and EchoCG- if clinical indications occurred; colonoscopy-

if clinical indications occurred; urethra and cervical smea examination- if clinical indications occurred).

As **THE CLINICAL VARIABLES** the folloing list of signs and symtoms(more or less sensitive/specific for SPA according the data provided by European SPA Study Group 1991) for assessment of study population:

1. Inflammatory spinal pain -- history or present symptoms of spinal pain in back, dorsal or cervical region with at least 4 of following: a/onset before age 45;b/ insidious onset;c/improved by exercise;d/ associated with morning stiffness;e/at least 3 months duration.
2. Anterior ches wall pain.
3. Alternating buttock pain-- past or present pain alternating between right and left gluteal regions.
4. Chest expansion >2, 5 cm
5. Reduction in spinal mobility-- Smyth`s test.
6. Synovitis-- past or present asymmetric arthritis or arthritis predominantly of low limbs.
7. Peripheral joint involvement pattern:mono-, olygo-, polyarticular;temporo-mandibular arthritis;predominantly DIP arthritis; uni-, bilateral hip arthritis.
8. Dactilitis--simultaneously involved DIP, PIP and MCP joints of certain digit or toe.
9. Enthesopathy at any site -- past of present spontaneous pain or tenderness at examination of the insertion of the Achilles tendon or plantar fascia, Heel pain, symphisitis, pericoxitis, epicondylitis, humero-scupular periarthritis or rotator calf tendinitis.

Extraarticular manifestations

10. Eye: conjunctivities, uveitis (acute antrior, posterior, uni-, bilateral).
11. Skin: psoriasis (vulgaris, inverse, pustular, erythrodermic) diagnosed by a physician erythema nodosum; keratoderma blenorragica; positive pathergy test.
12. Mucosa: balanitis circinata, oral and vaginal scrotal ulcerations, aphtous stomatitis
13. Nail disorders: pitting, ridging, onycholisis.
14. Gastro-intestinal system: acute diarrhea -- episode of diarrhea occurring within one month before arthritis;

    inflammatory bowel disease -- past or present Cron's disease or ulcerative colitis diagnosed by a physycian and confirmed by radiographic examination or endoscopy
15. Genito-urinary system: nongonococcal urethritis or cervicitis occurring within one month before arthritis.

16. Cardiovascular system: aortitis, aortic valve insufficiancy, disturbances of heart conduction system, vasculitis/phlebitis.
17. Pulmonary system: apical fibrosis with /out cistes; aspergilosis.
18. Kidney: amyloidosis, Ig A-nephritis with /out chronic renal insufficiency.

Radiographic features and signs of their progression:

19. Sacroilitis: bilateral grade 2-4, or unilateral grade 3-4, according to the following radiographic grading system: 0=normal, 1=possible, 2=minimal, 3=moderate, and 4= ankylosis.
20. Spondylitis: lumbar, thoracal, cervical:
    early manifestations: juxtainsertional osteoparosis, squaring of vertebral body
    late manifestations: local bone erosions, sclerosis, new bone formation (syndesmophites), anterior wedging and dorsal kyphosis, "bamboo" spine, apophysial spinal arthritis, costovetebral arthritis, spondylodiscitis, cervical subluxation.
21. Neurological system:"cauda equina" syndrome.
22. General signes and symptoms: fatigue, weight loss, low/high grade fever, anemia.
23. Positive effect of nonsteroid antiinflammatory drugs (NSAID), or local therapy due to prolonged cortocosteroid injections.
24. Family history-- presence of any of the following: AS, P/PsA, AAU, ReA, IBD

Some of these signs and symtoms such as peripheral arhritis/synovitis, low back pain and/or sacrroiliiitis, enthesitis, conjunctivitis, urethritis/acute enteritis or IBD, skin, mucosal manifestations occur at the onset of SPA or develop over first year of natural history and might be considered as the **start-point or baseline clinical variables.** If the disease has a self-limiting course all of these manifestations have to be ended within 12 months from the onset. Otherwise, the residual clinical features occurred beyond this period in addition to newly developed over natural history should be considered as **adverse outcomes** of the disease or variables possibly depended upon some baseline data(risk factors).

Adverse outcomes(AO) could be classified as immediate, intermediate and final types.

Immediate AO are those clinical variables(single or in combination) listed above that would persist after 12 month from disease onset and could be reversible or curable completely or at least slowly progressive and without any steady state organ/system impairment(anatomical defect) or disability(loss of function). In other words it might be cases of reactive seronegative arthropathy with delayed course or slowly progressing undifferentiated SPA cases.

Intermediate AO are the same clinical variables persisted and progressed to definite SPA entities(according to well established diagnostic criteria) in combination with newly developed signs and symptoms which might be appeared at the advanced stage of the disease(erosive arthritis, sacroiliitis uni-, bilateral above grade 2; radiologically confirmed spondylitis as well as systemic manifestations of SPA like aortitis, heart conduction system disturbances, lung fibrosis etc). Overally, intermediate AO could be characterized as the set of clinical variables of SPA natural history stage where irreversible impairment of any site of spine or/and peripheral joint involvement could occur, but the loss of their functional capacity does not excess 1-2 ARA functional class and/or there is no advanced functional insufficiency of any extraarticular organ/system involved. Patients with undifferentiated SPA also could have intermediate AO if the steady state signs and symptoms of impairment of any organ/system involved will occur without substantial loss of functional capacity.

Final AO are the clinical manifestations of functional 3-4 ARA class incapacity of locomotor apparatus as well as of advanced functional loss of other organ/system involved (cardio-vascular, pulmonary, urinary, digestive nervous etc). In other words, these variables are the measures of disability. Functional index scores of activities of daily living will be used for assessment of locomotor disability .

The structure of clinical variables occurred at the end stage of disease which lead to disability also will be studied: A/1. spinal ("Bamboo" spine/dorsal Kyphosis; microfractures of vertebral body; atlanto-occipital dislocation; rigid chest wall/chest expansion less than 2 cm);2 .peripheral joints (hip damage required uni-, bilateral hip replacement; temporo-mandibular joint ankylosis; arthritis mutilance of digit/toe; fibrose contracture or ankylosis of any joint; hind spurs)

B/extraarticular: 1. aortic valve insuffisiecy and/or conduction disturbances at the congestive heart failure stage; 2. renal amiloidosis and Ig A nephritis at the chronic renal insufficiecy stage; 3. apical pulmonary fibrosis at the chronic breath insufficiency stage; 4. complications of IBD lead to nutritional disturbances, anemia, weight loss; 5. neuropathy and/or "cauda equina" syndrome; 6. sequel of uveitis lead to visual loss.

Socio-economic variables to be used for assessment of handicap: employment status; change of occupation due to disease; work loss days per year; change in income; change of marrital status due to disease; expenditures related to health status maintainance.

Age/gender adjusted mortality rates of SPA patients, as well as death causality variables will also be analyzed.

Apart from concepts describing the stages of natural history of the disease, there is a need in concepts chracterizing the process of the disease development like severity and progression rate.

Severity or degree of expression of disease manifestations would be determined due to: 1. quantifying and scoring combinations of clinical and laboratory findings of interest at the entry visit examination and further annually shots over follow up; 2. estimating variables characterizing the process or clinical course such as number of exacerbations per year;duration of arthritic attacks and extraarticular flaws;effect of routinly used NSAID on them; number of unscheduled visits to rhematologist or hospitalisations (nights spent at the hospital) per year due to disease. In this study, articular index as a measure of disease severity will also be used.

Disease **progression rate** might be cosidered in context of severity and reflects the dynamics of annually measured incidence trends of of changings in earlier revealed and constantly persisting clinical variables as well as subsequently developing later manifestations (complications) of SPA. As the general measure of progression, the incidence of commonly accepted SPA entities developed from undifferentiated SPA over follow up and the incidence of final AO could be used.

As a measures of clinical variables, proportion of each one would be calculated as well as their incidence and cumulative incidence rates(and incidence of changings or progression rate for certain clinically and radiologically determined variables) over natural history.

Unfortunately, there are no systematic and comprehensive data regarding the usefulness of these clinical variables for characterisation of concepts like adverse outcomes, severity and progression rate at SPA. One of the objectives of proposed study is also preliminary testing and scoring of clinical variables(deviced earlier as diagnostic criteria) in terms to fulfill new needs. In other words, the aim is to create precise list of clinical variables that could characterize these concepts at appropriate level of sensitivity and specificity.

DATA COLLECTION

As mentioned above, the first step of study should be the listing of index cases or patients with any definite SPA entity registried in each HC facility of Yerevan and conducting abbreviate family history survey aimed to reveal eligible SPA families, then to carry out family study survey among FDRs of index cases. The newly identified FDRs with 50 per cent probability to contract SPA, then would be examined according to SPA diagnostic criteria (Amor, 1991). Finally, only the patients fullfiled them would be taken for a long term prospective follow up at the primary study sites (50 outpatient clinics, where rheumatological cabinets already exist). At the entry visit, in addition to demographic and anamnestic data already obtained via survey, the comprehensive physical examination would be performed including complaints, clinical tests for revealing sacroiliitis and enthesopathy; measurements of spinal movement and chest expansion (spondylometry, Schober's test, Smyth's test),

occurence of peripheral artritis/synovitis (swelling, tenderness, range of motion), as well as detail examination of extraarticular organ/systems (cardiovascular, pulmonary, digestive, genito-urinary, nervous).

Blood samples would be taken for HLA; RBC-isoantigen typing; serologically revealing antibodies against Chlamidia, Salmonella, Klepsiella, Campilobacter, Shigella, Yersinia, antigens, as well as antibodies cross-reactive with HLA B-27 (anti IgA, anti IgG, anti IgM, antisera would be used simulteniously for determining recent/ past infection process).

Routine laboratory blood, urine analyses would also be performed for identification of acute phase inflammatory variables.

Bacteriologic tests for fecal analysis aimed to reveal pathogens and possible disbacteriosis, as well as bacterioscopy of uretral smeas would also be performed (if indicated).

Radiographic evaluation of the SI joints and spine, as well as peripheral joints, feet would be performed at entry visit, if there is a strong clinical evidence of their involvement in inflammatory process.

Other instrumental diagnostic tools such as ECG, EchoCG, or colonoscopy would be used initially if there are clinical signs and symptoms (or at least anamnestic evidence) of possible involvement of these organ/systems.

The medicamental treatment of these patients would be limited only with NSAID and periodically (if indicated) local corticosteroid injections at the inflammatory sites. An exersise and active life style would be recommended all of them.

In general all of these patients would be checked up annually due to scheduled shots if there is no any substantial worsening in the health status between the shots. Possible unscheduled examinations might be linked to the exacerbations or reccurent arthritic attacks developed at these patients. Such patients would be considered as a group of severe cases or poor prognosis and the referral to second level of rheumatological servise might be needed for more attentive examination at the hospital. At the same time, variables like frequency of flaws, number of hospitalisations or unscheduled visits due to worsening of health status could be used as variables of poor prognosis or severity. Patients will have to be awared about the necessity to attend s/his rheumotologist if there is any (even not severe) worsening of health status or development any unknown earlier sign/symptom. It might be resonable touse as an insentive free of charge distribution NSAID for pations with severe disease course.

Next scheduled shots would be planning annually over 5-8 year follow up. Over each shot the following activities would be included: the same spectrum of physical examination or more widening the usage of instrumental diagnostic tools (especially radiography). It would be necessary to repeat padiographic films of earlier involved sites of spine SI and peripheral

arthritic joints for assessment of the evidence of possible progression. At the same time the sites of axial and extremital skeleton also needs for radiography if their involvement is established clinically or suspected via anamnestic data obtained between shots. The same concept is applicable for other diagnostic tools. However, even if there is no any clinical evidence of the involvement, it is resonable to examin radiologically or ECG/Echo organ/systems over late shots when the development of the clinically silent complications might be occurred with the increased probability.

At the late shots it is also necessary to perform more attentive examination of functional capacity loss not only of locomotor apparatus, but extraarticular organ/systems for more precise evaluation of incidence rate of study variables. There is also an urgent need in persistent analysis of attritional rate and possible causes of attrition aimed to control selection bias and to avoid the loss of patients under the follow up via escaped death cases. Each case of missing shot must be verified by the data clerk at the primary study site. Visits to municipal "Marriage, birth, death registry office" will have to be performed periodically. The computerised tracking system would be used for identifying persons at high risk of missing scheduled shots.

Meanwile reliability and validity of data presented by primary and secondary study sites will be cheked by members of Coordinating Group in terms to reveal inter-, intraobserver data differences, as well as analysed currently in accordance with data analysis plan. If needed an appropriate corrections of study design or daily activities of study sites will be implemented.

Concerning the time period for patients recruement under follow up, 2-3 years might be enough assuming that mean SPA incidence rate for Caucasians would be equal 15-20 per 100000 per year at population size of Yerevan under 10-45 is about one million. If over planning time period an appropriate cohort would not be recruited, next year recruitment could be used or sampling of yet healthy HLA B 27 (+) FDRs would be performed (what is also important to control possible selection bias).

Certain scientific research facilities of Yerevan (Hematologic Center, Microbiology, Immunology Institute) would be engaged for performing HLA, RBC isoantigen typing, as well as serological and immunological examinations needed.

DATA ANALYSIS PLANNING AND STATISTICAL INFERENCES

The results of HLA-A, B, C, DR and RBC isoantigen system typing would be analyzed in terms of their distribution among the study population, calculation of genotype haplotype and fenotype frequencies and then matching with the presence or absence of the appropriate inviromental pathogens. Only those combinations occurred with statistically

significant frequencies (revealed via univaried analysis) would be used for further comparison with possible adverse outcomes developed over natural history of the disease.

The clinical variables listed above would be tested or matched regarding time of onset (incidence) ; expressiveness (mono-, pauci- vs polyarticular involvement; degree of spinal involvement; duration of morning stiffness etc.); speed of changings (progression rate); frequency of relapses (number of hospitalisations/nights spent or loss of work day); statistically significant covariations of variables; association of study variables with certain final adverse outcomes of the disease; the effect of commonly used NSAID treatment regimens over them.

Such a data analysis plan implementing prior to final statistical inferrences would permit to reveal their usefulness as characteristic criteria for concepts like immediate, intermediate and final adverse outcomes, as well as severity and progression rate of the disease and thus would reduce number of variables to be compared.