Ankylosing Spondylitis, HLA-B27, *Klebsiella* and "Popper Sequences"

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Abstract: The cause of ankylosing spondylitis (AS) was investigated through 11 Karl Popper sequences. "Popper sequences" provide a powerful method of investigating a scientific problem. A "Popper sequence" consists of a "problem", "tentative theory", "error elimination" which then leads to a "new fact". The 11 "Popper sequences" establish that: (1) HLA-B27 lymphocytes injected into a rabbit evoke antibodies against *Klebsiella*, (2) anti-HLA-B27 tissue typing sera bind to *Klebsiella* antigens, (3) total serum IgA is elevated in AS patients, (4) antibodies to *Klebsiella* are present in AS patients from 16 different countries, (5) antibodies to *Klebsiella* in AS patients are disease specific, (6) *Klebsiella* bacteria can be grown from fecal cultures, (7) the sequence QTDRED found in HLA-B27 resembles a sequence DRDE found in pullulanase-D enzyme of *Klebsiella*, (8) *Klebsiella* pullulanase-A contains a sequence which resembles type I, II, and IV collagens, (9) sera from AS patients have cytopathic properties against sheep red cells coated with the cross-reacting peptides found in *Klebsiella* and HLA-B27 sequences, (10) *Klebsiella* bacteria grow preferentially on carbohydrate substrates, and this could be used to decrease bowel bacteria which may lead to a reduction of inflammatory parameters, (11) post-pubertal hormonally-induced muscle mass leads to increased starch consumption and onset of AS. The use of "Popper Sequences" suggests that *Klebsiella* microbes are the probable causative agents of AS.

Keywords: Ankylosing spondylitis, *Klebsiella,* HLA-B27, Popper sequences.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic and progressive disease characterized by inflammatory arthritis and eventual immobility of a number of joints, mainly involving the spine and related spinal structures.

It is a potentially disabling disease, and if left untreated, it could have a significant negative impact on the social and economic life of the affected patient [1].

One of the greatest discoveries in the history of rheumatology was the simultaneous demonstration by research workers from London and Los Angeles that some 96% of patients suffering from AS carried or belonged to the HLA-B27 group whilst the frequency of this marker in the general populations of the U.K or USA was around 8%. This great disparity between the frequency of this "major histocompatibility complex" marker, located on chromosome 6 and its low frequency in the general population raised the possibility that it could provide some insight in the causation of this disease. Patients with AS, often experience symptoms of this disease for several years before a final diagnosis is reached, and it was suggested that this could be called an early phase of AS [2, 3].

When early and late cases of AS patients are considered together they could account for up to 1% of the general population. It would appear that there are over 30 million individuals in the world, who are HLA-B27 positive and suffer from either AS or early stages of the disease.

Although it has been suggested that AS is an "auto-immune disease" the origin of the condition is to some extent unknown. The disease seems to start predominantly in the 20's to 30's age group and men appear to be affected more frequently than women.

It is proposed in this critical analysis to use the methods advocated by Sir Karl Popper to study the origin of this disease. Preliminary studies have shown that "Popper sequences" provide a powerful method of studying scientific problems such as rheumatoid arthritis [4, 5]. If the cause of AS can be found, then appropriate steps can be taken in the early stages of the condition so that both medical problems of the patients and financial costs to society can be minimised to the mutual benefits of both groups.

KARL POPPER AND SCIENCE

Popper proposed a new way of carrying out scientific research. He was a great critic of the Baconian myth that all science starts with observations and then slowly and cautiously proceeds to theories. There is a fundamental difference between "Baconian science" and "Popperian science". In "Baconian science" one does not speculate beyond one's data. However, in "Popperian science" one always speculates beyond one's data because this provides the questions which can be resolved by experimental investigations.

Popper emphasized that "science" starts with "problems" and not with "observations". It is the identification of the "problem" that starts a research worker speculating as how to arrive at a solution which will throw some light on the puzzle or question he is trying to answer. Without "problems to resolve", without "puzzles to elucidate" there is no science. In AS, the most interesting problem or puzzle has been the high frequency of HLA-B27 found in the patients.

Attempts to find the truth are not final, but open to improvement; and knowledge is conjectural, it consists of guesses or hypotheses rather than final and certain truths. Criticism and critical discussion with the help of experiments

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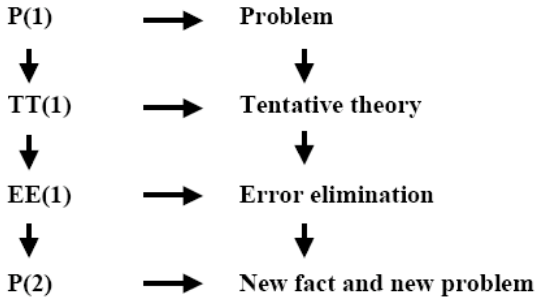
are our only means of getting nearer to the truth. It thus leads to the tradition of bold conjectures and free criticisms, the tradition which created the rational and scientific attitude of Western civilization.

Popper insisted that "the task of science is the search for truth", that is for true theories. Yet it is not the only aim of science. We want more than mere truth. What we are looking for is interesting and "deep" truth which has a high degree of explanatory power [6]. We require from a theory to be independently testable. Apart from explaining all the problems, it must have new and testable consequences of a novel kind; it must lead to the prediction of phenomena which have so far not been observed. Rheumatology is part of the scientific field that contains many interesting problems, and several unanswered questions that deal with the millions of the patients throughout the world who suffer from various musculo-skeletal problems. It is proposed to use Karl Popper's methods to try and study the problems of AS.

POPPER'S SCIENTIFIC METHOD

The Austrian-British philosopher of science, Sir Karl Popper (1902-1994) was born in Vienna, lectured in New Zealand and became Professor at the London School of Economics. He wrote many books including his classical "Logik der Forschung". Popper proposed that a scientific theory could not be proved but could be disproved or falsified. The theory that "All tigers are carnivorous" cannot be proved but is disproved by the observation of a single vegetarian tiger. Popper proposed a powerful analytical method to investigate scientific problems. The process can be described by the following simple schematic outline of how to tackle a scientific problem.

We start from a simple scientific problem (P1) and try to solve it by a "tentative theory" (TT1) which may or may not be correct. The theory will then be subjected to "error elimination" (EE1) either by logical criticism or experimental studies. As a result of these investigations a new fact, problem 2 (P2), will appear which in turn requires a scientific explanation. This is a "Popper sequence". If each "Popper sequence" generates new facts, then the original problem becomes richer in that it has more questions to resolve but at the same time the investigation gets closer to the truth of the inquiry, to the centre of the problem [7]. The sequence could be summarised as follows:



The second problem (P2) is different from the first , it is the result of the new situation that has arisen because of the tentative theories (TT1) and the error eliminations (EE1) which consist of logical analysis or criticism and experimental investigations suggested by the "tentative theories" (TT1). The new facts must then be explained by any new theory. Eleven "Popper sequences" have been identified in studying the problems of AS.

THE PROPERTIES OF THE PROBLEM OF AS

To investigate a "scientific problem" it is relevant to note the properties of the problem which define the puzzle or enquiry.

The properties of the AS problem would appear to be the following:

(1) Sex ratio: AS is found 3 times more frequently in men than women [8].

(2) Latitude: AS is found more frequently in populations living at high latitudes such as the Haida Indians and Inuit people of Canada [9] and are rarely found in populations near the equator such as the Bantu populations of Central Africa where AS is a rare disease [10].

(3) Early onset of AS: AS often starts in the teens or early twenties [11].

(4) Identical twin studies: AS is found more often in the second identical twin when the first twin has the disease [12, 13]. These identical twin studies suggest that there is an involvement of both genetic and environmental factors in the onset and pathogenesis of this disease.

(5) Genetic links: AS is found more frequently in individuals belonging to HLA-B27 [14, 15], where over 95% of AS patients belong to the HLA-B27 group whilst the frequency of these groups in the general population is about 8%. Therefore on average every eleventh person in the general population would appear to possess genes which predispose them to develop AS.

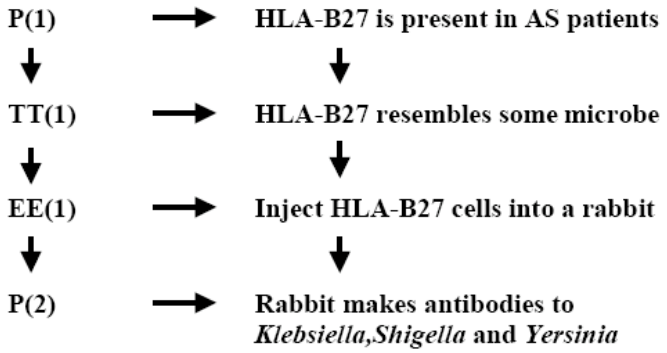
POPPER SEQUENCES

The following eleven Popperian sequences have been used to study the role of *Klebsiella* and cross-reactive self antigens in the aetiopathogenesis of AS.

First Popper Sequence

The link between AS and human histocompatibility antigens, particularly HLA-B27 was discovered by James and co-workers in 1973, from the Westminster Hospital in London using leucocyte cultures obtained from English AS patients [16] and also by Terasaki's group from Los Angeles in American AS patients [15]. It would appear that HLA-B27 is present in approximately 96% of AS patients whilst it is present in only 8% of English or American populations. Later this link between HLA-B27 and AS patients was confirmed in many populations throughout the world [17].

There are two ways of explaining this observation: either HLA-B27 molecules present some unknown antigens to immune cells or the HLA-B27 sequence itself resembles or shows "molecular mimicry" to some component of bacteria or viruses present in the environment. Since "molecular mimicry" appeared to work in rheumatic fever [18] we chose this approach to study the AS problem. This leads to the first Popper sequence.

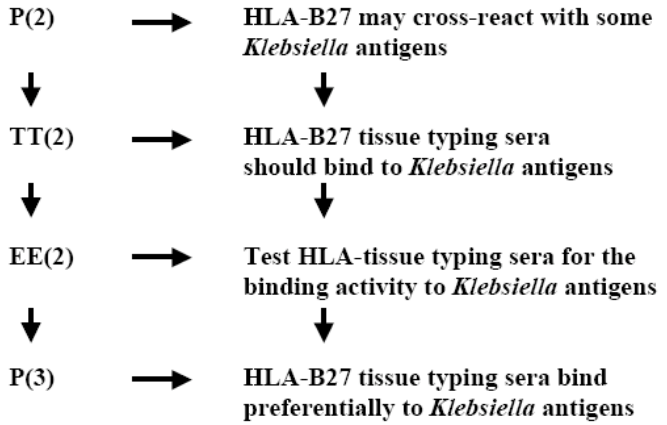


The result of the "First Popper sequence" suggests that Gram-negative bacteria such as *Klebsiella, Shigella* and *Yersinia* microbial species are somehow linked to HLA-B27 [19]. However, we do not know how specific is this observation? *Klebsiella* bacteria are commensal microorganisms and are frequently found in the bowel flora. *Shigella* and *Yersinia* microbes are often overt pathogens causing gastrointestinal symptoms such as abdominal pain and diarrhoea. In a radiobinding assay, elevated levels of antibodies to *Klebsiella* but not to *Shigella* were found in AS patients [20]. Furthermore, in a collaborative study with Finnish workers, there were no elevated levels of antibodies to *Yersinia* in English patients with AS when compared to Finnish patients suffering from *Yersinia* reactive arthritis [21].

The question arises if specific anti-HLA-B27 tissue typing sera can recognise some moiety on *Klebsiella* bacteria when compared to specific non-HLA-B27 tissue typing sera. This leads to the "Second Popper Sequence".

Second Popper Sequence

HLA antigens were initially identified by sera obtained from pregnant women and then calibrated against a panel of defined lymphocytes. The results of the "First Popper Sequence" indicate that when human HLA-B27 lymphocytes were injected into xenogeneic animals, antibodies were obtained against some Gram-negative bacteria including *Klebsiella*. The problem arises whether this observation is associated with human lymphocytes in general or can it be linked to the HLA-B27 specificity. This forms the basis of the "Second Popper Sequence".

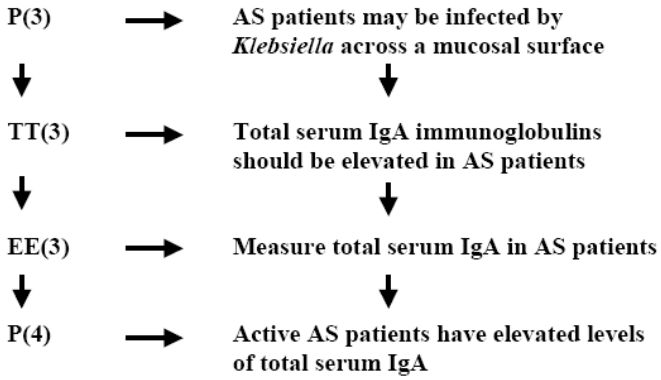


In this study, it was shown that only antibodies against HLA-B27 but no other MHC class-I molecules were significantly binding to *Klebsiella* microbes [22].

Third Popper Sequence

The possibility arises that AS patients may have been infected by *Klebsiella* bacteria, which are normal constituents of the bowel flora and are mainly located in the large bowel especially in the ascending colon.

The quantity of bacteria in the bowel is controlled by the "gut-associated lymphoid tissues" which respond to antigens of the luminal bacteria and other components especially of undigested food. When environmental antigens are acting across a mucosal surface this leads to an elevation of total serum IgA immunoglobulins. The question arises whether total serum IgA is elevated in AS patients during active phases of the disease, and this forms the basis of the "Third Popper sequence".



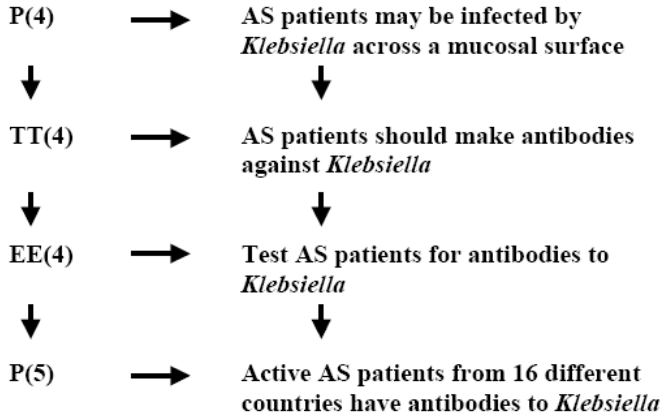
Various independent studies have shown that concentrations of total serum IgA immunoglobulins are increased significantly in active patients with AS [23, 24]. These results suggest that bowel bacterial antigens have been acting across a mucosal surface. If these antigens belong to *Klebsiella* bacteria, then AS patients should have elevated levels of antibodies against this microbe.

Antibodies to Gram-negative bacteria, such as *Yersinia, Salmonella* or *Shigella* are elevated in patients with "reactive arthritis"[25] and these usually involve the IgA immunoglobulin [26]. In some patients who have had an enteric infection with these Gram-negative microorganisms it is usually followed six to eight weeks later by the development of an inflammatory arthritis, especially in the large joints, such as the hips, knees and ankles. It is possible that *Klebsiella* infection or proliferation may not cause overt gastrointestinal symptoms but if antibody levels become elevated they may cause clinical symptoms and eventually lead to a disease like AS. This question leads to the "Fourth Popper sequence".

Fourth Popper Sequence

There appears to be a link between HLA-B27 and the commensal bowel microbe "*Klebsiella"*. Total immunoglobulin studies suggest that the environmental agent is acting across a mucosal surface because total serum IgA levels are elevated in AS patients. However, we do not know if AS patients have specific antibodies against this particular microbe. Antibody levels against *Klebsiella* microorganisms have been measured by a number of techniques: Widal agglutination [27], ELISA [28], immunoblotting [29], and immunofluorescence [30] (Table **1**). In each case, the results were similar, in that active AS patients had significantly

elevated levels of antibodies against *Klebsiella* micro-organisms.



The list of countries, year of the report and publication reference are as follows:

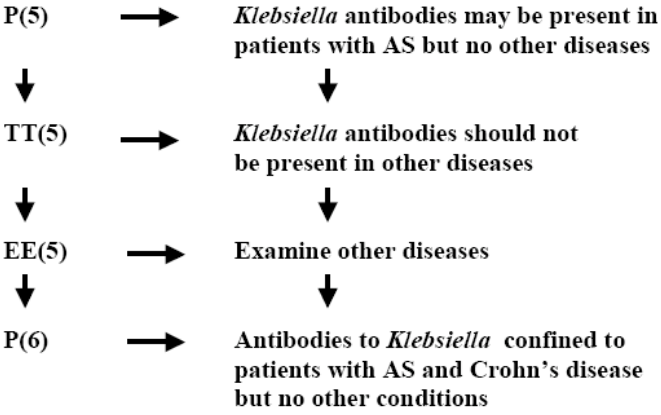
S. No.	Country	Year of Study	Reference
1.	England	1983	[31]
2.	USA	1987	[32]
3.	Scotland	1988	[33]
4.	Russia	1989	[34]
5.	Slovakia	1989	[35]
6.	China	1993	[36]
7.	Germany	1994	[37]
8.	Spain	1994	[38]
9.	Finland	1995	[39]
10.	Turkey	1996	[40]
11.	Japan	1997	[41]
12.	Sweden	1997	[42]
13.	Taiwan	1998	[43]
14.	Mexico	1998	[29]
15.	Netherlands	1998	[30]
16.	India	2002	[44]

Many studies have been carried out by different independent groups on antibodies against various microbial antigens in patients with AS. The high titre of anti-*Klebsiella* antibodies would appear to be specific because there was no such elevation in antibodies against many other microbial agents, especially Gram-negative microorganisms (Table **1**). The results of these studies have shown that there were no significant elevations in the levels of antibodies against other *Klebsiella*-related or unrelated microbes including Gram-negative enterobacteria such as *Salmonella* or *Yersinia* and Gram-positive streptococcus pathogenic bacteria. In 17 out of 27 studies anti-*Klebsiella* antibodies were shown to be present in AS patients at a statistically significant level ($p<0.001$), and in the other studies at lower levels of significance ($p<0.01$ or $p<0.05$). In one of the studies anti-*E.coli* antibodies were slightly elevated ($p<0.05$) but at the same time the anti-*Klebsiella* titre was clearly significantly elevated ($p<0.001$) in the AS patients [45].

Although AS patients have antibodies to *Klebsiella* in many different countries we still do not know how specific is this observation. Could elevated levels of antibodies to *Klebsiella* be present in other diseases? This brings us to the next "Fifth Popper Sequence".

Fifth Popper Sequence

An examination was carried out to measure the levels of antibodies against *Klebsiella* in the serum of active AS patients compared to patients with other chronic diseases, such as systemic lupus erythematosus and psoriatic arthropathy [46], as well as rheumatoid arthritis, ulcerative colitis and Crohn's disease [47]. However, apart from AS and Crohn's disease none of the patient groups with other rheumatic conditions had significant elevations in anti-*Klebsiella* antibody levels.



The presence of antibodies to *Klebsiella* bacteria in patients with Crohn's disease raises interesting issues in the pathogenesis of both AS and Crohn's diseases. It is possible that the same microbe is involved in both conditions, one in HLA-B27 positive individuals and the other one in HLA-B27 negative individuals. The next problem arises as to the mucosal site where these bacteria are located: Were they in the lungs, the gastrointestinal tract or somewhere else? This brings us to the "Sixth Popper sequence".

Sixth Popper Sequence

The location of the *Klebsiella* bacteria in active AS patients has so far not been resolved: Are these bacteria to be found in the respiratory tract or the gastrointestinal tract, two sites which have a mucosal surface and therefore would lead to an elevation in total serum IgA immunoglobulins, as shown in "Popper sequence 3". Antigens that act across a mucosal surface usually evoke predominantly IgA, but also IgG antibodies. It is possible that the *Klebsiella* might act across a mucosal surface.

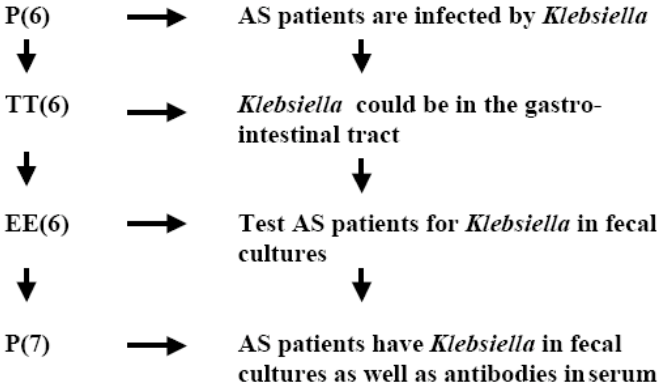


Table 1. Significantly Increased Antibodies Against *Klebsiella* and/or Cross Reactive Self Antigens in Ankylosing Spondylitis (AS) Patients Compared to Healthy Controls (HCs) Observed by Two Decades of Independent Studies

	Year of study	Antigens	Method	No. of AS patients	No. HCs	P values	Reference No.
1	1983	WB: Kp	ELISA	65	57	<0.005	[31]
2	1984	WB: Kp; St; Ye; Pa	ELISA	107	110	<0.01	[65]
3	1987	SP: Kp; HLA-B27	ELISA	24	90	<0.001	[32]
4	1988	WB: Kp	ELISA	59	35	<0.001	[33]
5	1989	WB: Kp; Pm; Ye	ELISA	75	28	<0.005	[34]
6	1989	WB: Kp; Ec	ELISA	20	20	<0.05	[35]
7	1991	LPS: Kp; St; Ye; Ec; Pm; Cj; Bb; Ct	ELISA	99	100	<0.01	[66]
8	1992	SDS-Ex: Kp; Pm	IB	66	51	<0.001	[67]
9	1992	WB: Kp	ELISA	14	14	<0.05	[68]
10	1993	WB: Kp	ELISA	31	15	<0.001	[69]
11	1993	WB: Kp	ELISA	60	45	<0.001	[36]
12	1994	WB: Kp; Pm; Ec	ELISA	84	100	<0.001	[70]
13	1994	Caps: Kp (77 serotypes)	ELISA	41	95	<0.01	[37]
14	1995	SP: Kp-PulD; Kp-PulA; HLA-B27; Collagens I & IV	ELISA	97	25	<0.001	[53]
15	1995	SDS-Ex: Kp	ELISA	171	100	<0.0001	[39]
16	1996	WB: Kp	ELISA	40	40	<0.001	[40]
17	1997	WB: Kp; Pm; Ec & SP: Kp-pulD; HLA-B27	ELISA	52	50	<0.001	[71]
18	1997	LPS: Kp, St, Se, Ec, Sf;	ELISA	56	60	<0.05	[41]
19	1997	WB: Kp; Pm; Ec; 10 NBIs	ELISA	35	60	<0.001	[47]
20	1998	LPS: Kp, St, Se	ELISA	100	50	<0.001	[72]
21	1998	WB: Kp (7 serotypes)	ELISA	40	40	<0.001	[73]
22	1998	WB: Kp; Pm	IIF	34	34	<0.001	[30]
23	1998	Caps: Kp (3 serotypes)	ELISA	184	100	<0.001	[74]
24	1998	LPS: Kp	ELISA+IB	44	40	<0.0001	[29]
25	1998	WB: Kp; Pm; Ec; St; Se; Ye	ELISA	52	51	<0.001	[43]
26	2001	WB: Kp & Collagens I, III, IV & V	ELISA	36	26	<0.001	[28]
27	2002	LPS+OMP: Kp; Ec; St	ELISA	20	15	<0.005	[44]
			Total numbers	1706	1451		

WB: Whole bacteria; SP: Synthetic peptide; SDS-Ex: Sodium dodecyl sulphate-extract; LPS: Lipopolysaccharide; Caps: Capsule; OMP: Outer membrane protein; ELISA: Enzyme-linked immunosorbent assay; IIF: Indirect immunoflurescent; IB: Immunoblot.
Kp: *Klebsiella pneumonia*; St: *Salmonella typhimurium*; Se: *Salmonella enteritidis*; Ye: *Yersinia enterocolitica*; Pa: *Pseudomonas aerouginosa*; Pm: *Proteus mirabilis*; Ec: *Escherichia coli*; Ct: *Chlamydia trachomatis*; Cj: *Campylobacter jejuni*; Bb: *Borrelia burgdorferi*; Sf: *Shigella flexineri*; NBIs: Normal bowel inhabitants; Kp-PulD/PulA: *Klebsiella* pullulanase D and A enzymes.

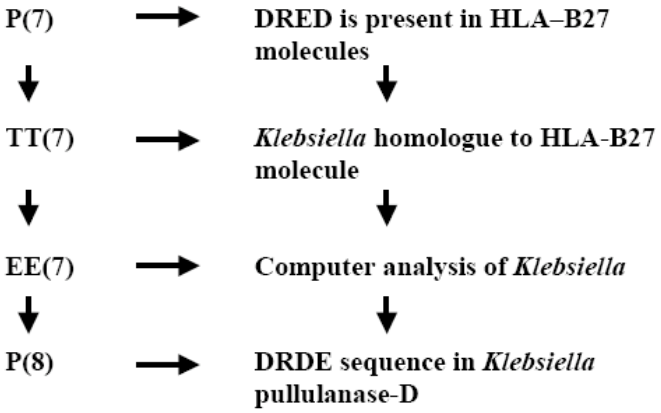
There appear to be some studies which show positive fecal cultures for *Klebsiella* in active AS patients [48, 49], another one with *Klebsiella* and other enterobacteria [50], although other groups have been unable to confirm these observations [51, 52].

These six "Popper sequences" have identified so far that HLA-B27 resembles some *Klebsiella* antigens. Furthermore, it would appear that antibodies to this microbe are present in AS patients, whilst no such antibodies are present in other chronic diseases and the site of infection would appear to be the gastrointestinal tract especially around the ileo-caecal junction and the ascending colon.

It appears that *Klebsiella* bacteria are clearly involved in AS patients but the pathological mechanism of joint damage and its link to HLA, requires further examination. We still do not know which component of HLA-B27 is associated with *Klebsiella* bacteria. This brings us to the "Seventh Popper sequence".

Seventh Popper Sequence

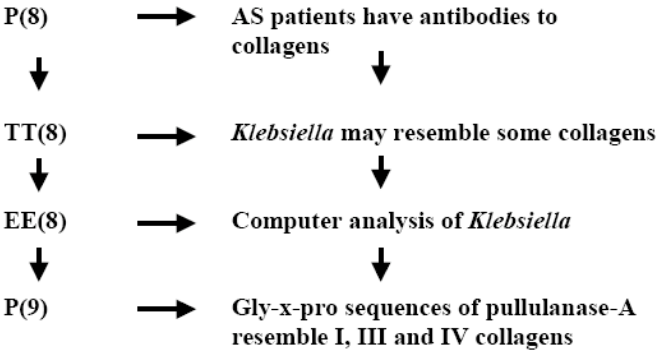
The enzyme "nitrogenase reductase", which is present in *Klebsiella* has a 6 amino acid homology with HLA-B27 [32]. This enzyme is not expressed in *Klebsiella* bacteria at a temperature of 37°C but is found mainly in soil bacteria at a temperature of 32°C. However, it is not clear whether QTDRED is somehow linked to other molecules in *Klebsiella* microbes.



The "error elimination" (EE7) in this Popper sequence consists of carrying out a computer analysis of the components of the *Klebsiella* bacteria to see if they resemble or show "molecular mimicry" to the QTDRED sequence. A sequence DRDE was found in pullulanase-D which showed molecular mimicry with DRED sequence present in HLA-B27 [53].

Eighth Popper Sequence

If the collagens present in the large joints are targeted by antibodies [53] then this suggests that any aetiological agent proposed for the cause of AS must also explain the geography or the anatomy of the disease. This leads to the next Popper sequence.

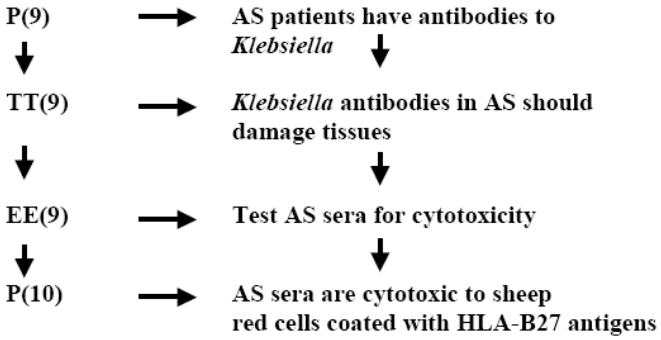


The chief site of pathology in AS is the lumbar spine and the large joints. Gly-x-pro repeating sequences have been found in pullulanase-A components of *Klebsiella* micro-organisms which show molecular similarity with type I, III and IV collagens and antibodies to collagens have been described in AS patients [53]. These collagens are found predominantly in the tendons, spine, large joints and uvea. Therefore antibodies to pul-A would have cytopathic properties against such collagens and would explain the distribution of pathological sites in AS.

Ninth Popper Sequence

AS is characterised by inflammatory episodes and their occurrence can be monitored by the presence of an elevated erythrocyte sedimentation rate (ESR) and/or elevated C-reactive protein (CRP) levels [54]. It is important here to distinguish between clinical assessments of pain as measured by subjective "analogue scales" whereby the patient indicates on a scale of 1 to 10, the degree of discomfort and pain felt, as opposed to an objective biochemical measurement of inflammation as given by an ESR or CRP. The usual levels which determine active disease and presence of biochemical inflammation is an ESR >30 mm/hour or a CRP level >15 mg/litre.

The crucial question arises whether such inflammatory episodes could be triggered by the high titres of anti-*Klebsiella* antibodies that have been identified in AS patients. The Popperian question arises whether such cytopathic effects can be demonstrated as an indication that anti-*Klebsiella* antibodies are the first triggers or agents in this disease. This will provide an answer to the important question "Do AS sera produce tissue damage?"



Sera from active AS patients were tested by a complement-mediated cytotoxicity assay. Sheep red cells were coated with HLA-B27 peptides or EQRRAA (HLA-DR1/4) or ESRRAL (*Proteus* haemolysin) peptides and compared to sera from patients with rheumatoid arthritis and healthy blood donors. AS sera were found to have cytotoxic activity against HLA-B27 coated peptides but not against peptides involved in rheumatoid arthritis (Fig. **1**) [55]. This brings us to the next Popper sequence.

Tenth Popper Sequence

In various independent studies throughout the world, AS patients were found to have elevated levels of antibodies to *Klebsiella* bacteria. Therefore, one possible way of reducing the numbers of *Klebsiella* microbes in the large bowel of AS patients is to reduce the quantity of substrates required for their growth.

Dietary starch provides an important substrate for the growth of gut microbes, including *Klebsiella*. In an experimental study, it was shown that rats fed potato starch had an increase in the bulk of intestinal enterobacterial microflora [56]. In another in vitro study, the mean number of *Klebsiella* per gram of substrate was found to be significantly higher following culture with 3 different sugars (glucose, sucrose and lactose) compared to its incubation

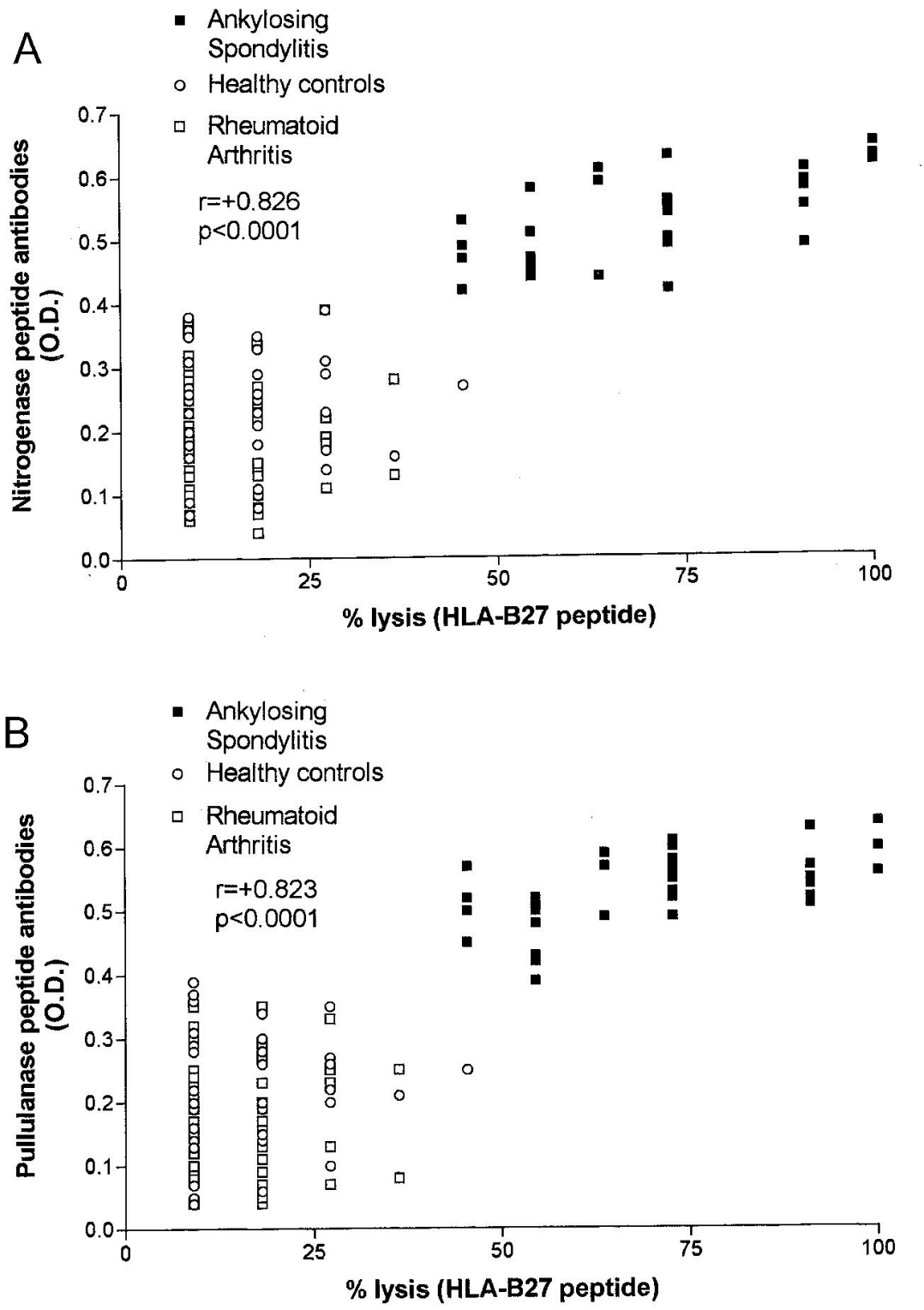


Fig. (1). Correlation of anti-*Klebsiella* nitrogenase (**A**), anti-*Klebsiella* pullulanase (**B**) IgG antibody levels for AS and RA patients and controls and percentage lysis of sheep red blood cells coated with HLA-B27 peptide. (*With permission, Wilson et al, 2003*).

with 11 different amino acids, thus showing that carbohydrates derived from starch are more efficient substrates for the growth and proliferation of *Klebsiella* [57]. Furthermore, Finegold and colleagues have observed that the number of *Klebsiella* and other intestinal microbes increased significantly in the fecal cultures obtained from 45 American vegan subjects taking high starch/low protein diet compared to 45 subjects consuming low starch/high protein diet [58].

A review of the literature showed that mono- and disaccharides were present in significant quantities (5-10gm/day) in ileostomy fluids [59]. Furthermore, up to 10%

of starch has been shown to escape the absorption from small intestine in healthy individuals, indicating that a sizable proportion of starch consumed daily may reach the large intestine [60]. This bulk of undigested starch forms an important substrate for the growth and proliferation of intestinal bacterial flora including also *Klebsiella*.

It would appear that the quantities of carbohydrate substrates entering the large bowel across ileo-caecal junction are important in determining the number of *Klebsiella* microbes. An important source of carbohydrate substrate comes from dietary starch. Dietary starch consists of approximately 20% amylose and 80% amylopectin. Amylose is a linear polymer consisting of α-(1-4) links between glucose residues and these can be readily hydrolysed by amylases present in digestive enzymes. However, amylopectin is a branched polymer, consisting of linear sequences of amylose like chains linked by α-(1-4) bonds but for every 10 to 15 residues, there is a α-(1-6) side chain giving rise to a branched structure. The problem is that digestive enzymes cannot break down α-(1-6) links present in amylopectin. The digestion of starch in the small bowel is limited by the inability of luminal digestive enzymes to break α-(1-6) bonds of amylopectin and giving rise to "hard starch" [61]. Its accumulation could help in the provision of substrates for growth and propagation of gut bacteria including also *Klebsiella* microbes. *Klebsiella* pullulanase is a powerful enzyme which degrades α-(1-6) amylopectin links, thereby providing more substrate for the growth of *Klebsiella*. This leads to the next Popper sequence.

P(10) → **Pullulanase enzymes can degrade "hard starch" and provide substrates for the growth of *Klebsiella***

↓

TT(10) → **Disease activity in AS patients should be reduced by decreased starch intake**

↓

EE(10) → **Test ESR in AS patients after a "low starch diet"**

↓

P(11) → **AS patients have a reduction in ESR following a "low starch diet"**

In a nine month study of AS patients on a "low starch diet", the mean ESR dropped, whilst there was no such reduction when the same patients were examined for 9 months before the onset of the diet intake [62]. Furthermore, in the same study, the total serum IgA dropped over the same period of time.

Eleventh Popper Sequence

Although it is also recognized among women, AS has often been referred to as a disease of young men. The onset of the disease is usually in the late teens to twenties and the male:female ratio has been estimated to be 3:1 [11]. This early onset of the disease and sex ratio differ markedly from rheumatoid arthritis where it usually starts in the forties and fifties age groups and occurs more frequently in women.

Any theory trying to explain AS must also account for this early age of onset and male predominance. So far we have proposed that the link with HLA-B27 leads to the identification of the bowel microbe *Klebsiella* which depends for its growth on the consumption of adequate amounts of dietary starch and this leads to the next Popper sequence.

P(11) → **Onset of AS starts frequently in teens and twenties, especially in men**

↓

TT(11) → **Onset may be linked to puberty, male hormones and muscle mass**

↓

EE(11) → **Examine whether men consume more calories than women**

↓

P(12) → **Men consume 40-50% more calories than women**

Ever since 1918, when Harris and Benedict from the Carnegie Institute in Washington [63] published their equation for determining daily calorie requirements, there have been numerous studies showing that at puberty and thereafter, nutritional requirements in men increase sharply due to the development of hormonally induced muscle mass. Before puberty girls and boys of the same age and height consume the same quantities of calories. After puberty, however, this will change where on average men require 40-50% more calories than women, the average daily recommended intake in women being 2,000 calories compared to 3,000 calories in men, depending on the degree of physical activity. Clearly the extra 40-50% calorie intake in some men may contain starch compounds which will contribute to an increased growth of *Klebsiella* bacteria near the ileo-caecal junction and ascending colon. This in turn will lead to the production of cytopathic antibodies which will attack tissue targets in the spine and elsewhere having cross-reacting antigens such as collagens I, III and IV as well as HLA-B27.

It would appear that the molecular mimicry theory involving HLA-B27 and *Klebsiella* can provide an explanation for the early onset of AS in young men.

DISCUSSION

The probable cause for the onset and development of AS has been identified through the demonstration of eleven "Popper sequences", each of which provided a new fact about the disease. If any other theories were to be suggested, it would have to account for the "new facts" uncovered by this Popperian analysis.

Current therapy of AS involves the use of agents such as sulphasalazine, methotrexate and anti-tumour necrosis factor (TNF) biologicals which significantly reduce the degree of inflammation [64]. The discovery that AS is triggered or caused by *Klebsiella* in the large bowel opens a new approach to the treatment of this disabling arthritic disease.

In science, we are trying to get closer to the truth but in medicine, the results of our investigations should help the patient as suggested by the Hippocratic Oath. Sufficient evidence has been uncovered using Popperian methods of

scientific research for anti-*Klebsiella* therapy to be assessed in the management of AS.

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