serological tests in venereal syphilis

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. J. SPERNA WEILAND
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 18 FEBRUARI 1981 DES NAMIDDAGS
TE 3.45 UUR

DOOR

alfred notowicz

GEBOREN TE AMSTERDAM

1981
PALLAS-OFFSET N.V. - DEN HAAG

PROMOTOR : PROF. DR. E. STOLZ
CO-REFERENTEN: PROF. DR. M.F. MICHEL
PROF. Dr. H.A. VALKENBURG

CONTENTS*

Chapter 1	Introduction	1
Chapter 2	Syphilis. Clinical and therapeutic aspects	2
2.1.	Introduction	2
2.2.	Pathogenesis	2
2.2.1.	Causative microorganism	2
2.2.2.	Mode of infection and infectiosity	3
2.3.	Histopathology	4
2.4.	Specific defence	4
2.5.	Subdivision of venereal syphilis	5
2.6.	Early infectious syphilis	6
2.6.1.	Primary syphilis	6
2.6.1.1.	Incubation period	6
2.6.1.2.	Localization	6
2.6.1.3.	Characteristics	6
2.6.1.4.	Differential diagnosis	6
2.6.2.	Secondary syphilis	7
2.6.2.1.	Incubation period	7
2.6.2.2.	Characteristics	7
2.6.2.3.	Diagnosis	8
2.6.2.4.	Differential diagnosis	8
2.7.	Early and late latent syphilis	8
2.8.	Late syphilis (tertiary syphilis)	9
2.8.1.	Gummatous syphilis	10
2.8.1.1.	Differential diagnosis	11
2.8.2.	Cardiovascular syphilis	11
2.8.2.1.	Clinical symptoms	11
2.8.3.	Neurosyphilis	11
2.8.3.1.	Diagnostic procedures	11
2.8.3.2.	Asymptomatic neurosyphilis	12
2.8.3.3.	Late signs of symptomatic syphilis of the nervous system	12
2.9.	Pregnancy and syphilis	13
2.9.1.	Introduction	13
2.9.2.	Mode of infection	13

* Tables and figures are placed at the end of each chapter

2.9.3.	Therapeutic approach of the pregnant patient	13
2.9.4.	Conduct at delivery and during the first months of life of an infant with possible congenital syphilis	14
2.9.5.	Consequences of maternal syphilis for the foetus	15
2.9.6.	Diagnosis of syphilis in neonates	15
2.9.7.	Early congenital syphilis	15
2.9.8.	Late congenital syphilis	15
2.9.8.1.	Stigmata of late congenital syphilis	16
2.10	Therapy	16
2.10.1.	Side effects of treatment with penicillin	18
Chapter 3	Serological tests in syphilis. A general survey	19
3.1.	Introduction	19
3.2.	Non-treponemal tests	19
3.2.1.	Complement fixation tests	19
3.2.1.1.	Anti-complementarity	20
3.2.2.	Flocculation tests	20
3.2.3.	Biologically false-positive tests	21
3.3.	Treponemal tests	21
3.3.1.	Group-specific treponemal tests	21
3.3.1.1.	Reiter protein complement fixation test	21
3.3.1.2.	Reiter protein counter-immuno-electrophoresis test	22
3.3.2.	Type-specific treponemal tests	22
3.3.2.1.	Treponema pallidum immobilization test	22
3.3.2.2.	Fluorescent treponemal antibody absorption test	23
3.3.2.3.	Fluorescent treponemal antibody absorption IgM test	24
3.3.2.4.	Treponema pallidum haemagglutination assay	25
3.3.2.5.	Enzyme immuno-assay	26
Chapter 4	Aims of the study	27
Chapter 5	Material and methods	28
5.1.	Diagnostic criteria	28
5.1.1.	Primary syphilis	28
5.1.2.	Secondary syphilis	28
5.1.3.	Early latent syphilis	28
5.1.4.	Early infectious syphilis	29
5.1.5.	Late latent syphilis	29

5.1.6.	Cardiovascular syphilis	29
5.1.7.	Symptomatic and asymptomatic neurosyphilis	29
5.1.8.	Endemic treponematosis	29
5.1.9.	Congenital syphilis	30
5.1.10.	Syphilis - not further classifiable	30
5.1.11.	Possibly syphilis	30
5.1.12.	No syphilis	30
5.1.13.	Sero-resistance	30
5.2.	Serological testing and laboratory techniques	31
5.2.1.	Serological testing	31
5.2.2.	Laboratory techniques	31
5.3.	Treatment	32
5.3.1.	Civilians	32
5.3.2.	Sailors	33
5.3.3.	Alternative therapies	33
5.4.	Methods used in collection, registration and processing of data	34
5.5.	Statistical methods	35
5.6.	Graphic representation	36
5.7.	Patients	36
5.8.	Follow-up of treated patients	37
Chapter 6	The course of various non-treponemal and treponemal serological tests when an infection with T. pallidum is left untreated	43
6.1.	Introduction	43
6.2.	Material and methods	45
6.3.	Results	45
6.3.1.	Percentages of positive tests in untreated patients with syphilis in various stages	45
6.3.2.	Graphic representation of the course of serological syphilis tests when an infection with T. pallidum is left untreated	46
6.4.	Discussion	47
6.5.	Conclusions	50
Chapter 7	Influence of penicillin treatment on the course of various non-treponemal and treponemal serological tests in patients with acquired syphilis	67

7.1.	Introduction	67
7.2.	Material and methods	69
7.3.	Results	70
7.3.1.	Graphic representation of the course of various serological syphilis tests in response to penicillin treatment, regardless of the dosage and duration of treatment	70
7.3.1.1.	Sero-changing primary syphilis	71
7.3.1.2.	Sero-positive primary syphilis	71
7.3.1.3.	Secondary syphilis	72
7.3.1.4.	Early latent syphilis	73
7.3.1.5.	Late latent syphilis	73
7.3.2.	Comparison of patients with early infectious syphilis treated with different penicillin dosages	74
7.4.	Discussion	74
7.5.	Conclusions	78

Chapter 8	Screening tests. Effectiveness of various serological tests or test combinations used in The Netherlands for demonstration or exclusion of infection with T. pallidum in various populations	94
8.1.	Introduction	94
8.2.	Material and methods	95
8.3.	Results	97
8.3.1.	Patients with a negative VDRL/RPCF combination	97
8.3.2.	Patients with a negative VDRL/Kolmer combination	97
8.3.3.	Patients with a negative VDRL/FTA-ABS combination	98
8.3.4.	Predictive values of the Kolmer, VDRL, RPR, RPCF, FTA-ABS, TPI and TPHA tests	98
8.3.5.	Probability of syphilis in the case of a negative test result	98
8.4.	Discussion	98
8.5.	Conclusions	102
Summary		111
Samenvatting		114
References		117
Dankwoord		127
Curriculum vitae		128

ABBREVIATIONS

Kolmer	= Wasserman-Kolmer complement fixation-test
VDRL	= Venereal Disease Research Laboratory-test
RPR	= Rapid Plasma Reagin card-test
RPCF	= Reiter Protein Complement Fixation-test
FTA-ABS	= Fluorescent Treponemal Antibody-Absorption-test
TPI	= Treponema Pallidum Immobilisation-test
TPHA	= Treponema Pallidum Haemagglutination Assay
dub	= dubious
ac	= anticomplementary
tot	= total
trep	= treponemacid
tf	= test failed
nd	= not done
Me	= median
$X_{0.25}$	= 25th percentile (1st quartile)
$X_{0.75}$	= 75th percentile (3rd quartile)

CHAPTER 1

INTRODUCTION

Apart from identification of the causative microorganism, serological blood testing is still the principal aid in the diagnosis of venereal syphilis. In latent syphilis it is in fact the only diagnostic aid. In the diagnosis of late symptomatic syphilis, additional organ-specific diagnostic procedures are indispensable.

Interpretation of the results of serological syphilis tests often poses problems in actual practice. Apart from possibly inadequate knowledge of the natural history of the disease and the course of serological syphilis tests, this may be due to the fact that a fairly large number of different tests are in use in The Netherlands. Often, moreover, a single laboratory result is expected to give a definite diagnosis.

There are numerous views and recommendations with regard to the treatment of syphilis. Recent studies on an international level have shown that no definite conclusions can as yet be formulated in this respect (Luger, 1968; Idsoe et al., 1972; Thompson et al., 1976).

In the dermato-venereological department of the Rotterdam University Hospital, well-documented case records of syphilis patients were available. Several different serological tests had been performed in these cases, both before and after treatment. It was therefore possible - within the context of the research project 'Interaction between Man and Treponema pallidum' now in progress in the department - to carry out a retrospective study of the results and interpretation of serological tests both before and after treatment in groups of syphilis patients. Specifically, it was possible to compare a relatively large number of different tests within a given group of patients.

Chapter 2 and 3 present a survey of clinical symptoms, the treatment of syphilis, and general aspects of serological tests in current use.
Chapter 4 through 8 present and discuss personal observations.

CHAPTER 2

SYPHILIS. CLINICAL AND THERAPEUTIC ASPECTS*

2.1. Introduction

There are several theories on the origin and dissemination of syphilis. One of these theories holds that Columbus and his crew brought syphilis back from the New World to Spain and then to Naples, whence it spread over Western Europe. Others maintain that syphilis was already present in Europe at that time. The disease was originally known as lues venera. The term syphilis, now most widely used internationally, was introduced in the 16th century by Ricord and derives from a 16th-century poem about the shepherd Syphilus ('friend of swine'), who was suffering from evil sores.

2.2. Pathogenesis

2.2.1. Causative microorganism

Syphilis is the principal representative of the diseases caused by micro-organisms of the species Treponemata. Syphilis (synonym lues) is caused by Treponema pallidum (T. pallidum). Other treponematoses are framboesia (yaws), caused by T. pertenue, and pinta, caused by T. carateum. These treponemata are not distinguishable either morphologically or serologically but it is possible to demonstrate the development of distinctly different characteristic changes in animal experiments. Other treponemata are saprophytes: T. macrodentium and T. microdentium on the oral, and Borrelia refringens on the genital mucosa. The presence of these saprophytes can impede laboratory investigation of ulcers. The causative microorganism of syphilis, T. pallidum, is a very delicate, spiral- or corkscrew-shaped organism which varies in length from 8 to 19 μ. In a native physiological saline preparation, both the shape and the movements of the microorganism can be visualized by dark-field or phase-contrast microscopy. Three characteristic movements are distinguishable:

---

* The text of this chapter is a rendering, with a few additions and corrections, of the Dutch text of the chapter on 'Syphilis' which the author wrote for the textbook on dermatological and venereal diseases, Huid- en Geslachtsziekten, Bohn, Scheltema & Holkema, Utrecht 1980. The publisher has kindly permitted the author to use this text.

rotation on the longitudinal axis, a forward movement in the direction of the longitudinal axis, and flexible lateral movements.
In tissue sections, treponemata can be demonstrated with the aid of a silver stain (Whartin and Starry, 1921). Direct and indirect immunofluorescence techniques can also be used to demonstrate treponemata in tissues and body fluids (cerebrospinal fluid, vitreous fluid, endolymph) (Wilkinson and Cowell, 1971).
In its active phase, the organism divides itself transversely about once every 30 hours. It is assumed that, after a prolonged presence of the organism in the host, this interval between divisions can be markedly increased: up to several days (Rosahn and Rowe, 1950). The number of treponemata presumably increases gradually during the infection, well into the second stage, whereupon it decreases. Outside the body, T.pallidum can survive only a few hours. Attempts to cultivate T.pallidum on an artificial medium have so far failed, but cultivation by inoculation of test animals (rabbit, monkey) is possible. Under very special conditions (anaerobic environment), T.pallidum can live in artificial nutrient media for a few days without dividing.

2.2.2. Mode of infection and infectiosity

Infection generally takes place via intimate physical contact, of which sexual intercourse is the example par excellence. T.pallidum must be present on the skin or mucosa of the contact involved. Another prerequisite for infection is that the treponemata can pass the epidermal barrier. This poses no difficulty at the sites of epithelial defects and at sites normally devoid of epidermis: the mucosa. It is generally believed that the disease is no longer a localized condition a few hours after infection.
Physicians and medical personnel can be accidentally infected via a skin lesion after contact with blood from a patient with infectious syphilis. Syphilis can also be transmitted by blood transfusions using fresh blood from persons suffering from early syphilis.
Although it is theoretically impossible to define limits of infectiosity, it is assumed that only untreated early syphilis (i.e. untreated syphilis within 2 years of the infection) must in practice be regarded as infectious. An exception to this rule is untreated syphilis in a pregnant woman: when no adequate treatment has been given, infection of the foetus in utero is possible even more than 2 years after the infection (Stokes et al., 1941).

2.3. Histopathology

The fundamental histological changes in syphilis are localized in and around the blood vessels; in particular there is a predominant perivascular infiltrate of lymphocytes and numerous plasma cells, and endarteritis and endophlebitis. In tertiary syphilis (gummatous forms) one finds in addition a tuberculoid infiltrate with caseation, which may cause a close resemblance to tuberculosis. In such cases the vascular lesions and the plasmocellular infiltrate can be helpful in differential diagnosis.

2.4. Specific defence

Humoral:

Even in the sero-negative stage of syphilis, an increased serum immunoglobulin concentration can be observed which attains its maximum during the secondary stage. The principal contribution to this increase is made by immunoglobulins of the IgG and IgM classes. Penicillin treatment during 6 weeks causes a return of immunoglobulin levels to virtually normal values (Menke, 1975). An increased IgE level, too, has been demonstrated in early syphilis (Green et al., 1976; Bos et al., 1980a).
The presence of antibodies against T. pallidum can be demonstrated with the aid of several serological tests. There are indications that several nonspecific antibodies are synthesized during an infection with T. pallidum, as demonstrated by an increased presence of rheumatoid factor and antibodies against the patient's own erythrocytes (Doniach, 1976). Moreover, fluorescence has been described in various tissue substrates, including epidermal tissue, with sera from patients with early syphilis (De Jong et al., 1978).

Cellular:

When a purified extract of pathogenic treponemata is intracutaneously injected, a skin reaction may develop which, in view of the chronological course and the histological characteristics, can be plausibly regarded as a delayed-type hypersensitivity reaction. Patients with early syphilis nearly always show a negative reaction, whereas the reaction in those with late syphilis is often positive (Csonka, 1950; Huriez et al., 1961). Patients with late symptomatic syphilis likewise frequently show a positive reaction, with the exception of those with dementia paralytica, whose skin reaction is negative. In the latter patients the serological tests show high titres and vast numbers of treponemata are found in the body

(Stokes et al., 1941). It has also been found that neonates with congenital syphilis - unlike healthy neonates - could not be sensitized by means of the obligate sensitizing agent dinitrochlorobenzene (Parent and Smythe, 1973).

Histological examination of lymphoid organs has revealed lymphocyte depletion in the paracortical areas of lymph nodes in patients with early syphilis and in the periarteriolar sheaths in the spleen of children who died from congenital syphilis. These areas in lymph nodes and spleen are thymus-dependent areas in which mainly T-lymphocytes are normally localized (Levene et al., 1971; Turner and Wright, 1973). Moreover, there are indications of a decrease in the number of circulating T-lymphocytes in primary syphilis (Bos et al., 1980b). There is as yet no agreement about the question whether the in-vitro phytohaemagglutinin (PHA) response of lymphocytes from patients with primary and secondary syphilis is or is not diminished as compared with that in normal controls. Equally controversial is the question whether plasma from these patients is able to suppress the PHA response of lymphocytes from healthy donors. It seems to be an established fact that lymphocytes from patients with early syphilis are less readily stimulated than those from normal controls when submitted to the influence of a protein preparation isolated from T. refringens, which is related to T. pallidum. From this fact it has been concluded that suppression of cellular immunity is involved, which remains limited to the treponemal antigens (From et al., 1976).

Considering the specific immunological defence, we are confronted with relatively early stimulation of the antibody-producing apparatus, leading to synthesis not only of antibodies against treponemal antigens but also of antibodies of 'deviant' specificity. Progression of the disease takes place in spite of the production of these antibodies. Cellular immunity, on the other hand, develops only late in the course of the infection, and several lines of research indicate the possibility of a disorder at the T-lymphocyte level during early syphilis.

2.5. Subdivision of venereal syphilis

The traditional division of syphilis into three stages - primary, secondary and tertiary syphilis - is in fact arbitrary, and the stages often overlap. Yet this subdivision is still widely used and found satisfactory in day-to-day practice. Another subdivision is that into early infectious syphilis (primary, secondary and early latent syphilis) and late syphilis (late latent and symptomatic syphilis).

2.6. Early infectious syphilis

2.6.1. Primary syphilis

2.6.1.1. Incubation period

The incubation period averages 17–28 days (extremes: 9 and 90 days).

2.6.1.2. Localization

The lesion is usually localized in the anogenital region or areas near the external genitals (pubic region, scrotum). Extragenital localizations– mouth, lips, fingers – are rare (De Koning et al., 1977). In some cases the patient is unaware of the ulcers due to their unusual localization, e.g. rectum, vaginal wall and cervix uteri. In these cases the inguinal lymph nodes are often not swollen either. This explains the fact that women and homosexuals who practice rectal intercourse, often present in a later stage of syphilis (usually secondary).

2.6.1.3. Characteristics

A generally painless papule with an eroded surface or an ulcer develops on the mucosa or at the site of a skin defect. The ulcer may be indurated, in which case it is called ulcus durum or hard chancre. Not infrequently, however, there are atypical lesions such as multiple superficial ulcerations of herpetiform appearance, erosive balanitis and, sometimes, markedly inflammatory oedema with paraphimosis. Reports in recent years have mentioned an increase in the incidence of atypical primary lesions, up to 40% of the total number of patients with primary syphilis (Notowicz et al., 1973). An ulcer without regional lymph node swelling is called primary lesion, but when it is associated with painless, palpably hard regional lymph node swelling, it is known as primary complex. Left untreated, the primary lesion heals within 3–8 weeks, sometimes leaving a slightly atrophic cicatrix.

2.6.1.4. Differential diagnosis

Possibilities to be considered in differential diagnosis include herpes genitalis, common forms of balanitis, scabies papules irritated by scratching, chancroid, lymphogranuloma venereum, donovanosis and common traumatic lesions. Carcinoma of the penis also is often associated with ulceration. In patients with a genital lesion, syphilis can be eliminated only by careful history taking (including family history if necessary),

clinical examination, repeated dark-field and serological studies and serological follow-ups over a period of 3 months.

2.6.2. Secondary syphilis

2.6.2.1. Incubation period

When primary syphilis is not identified and treated as such, the symptoms will nevertheless subside and finally disappear, but subsequently the symptoms of secondary syphilis can develop – on average 6 weeks after development of the ulcer (extremes: 1 and 6 months). A transitional form in which the symptoms of primary syphilis are still present, can also develop.

2.6.2.2. Characteristics

The most general symptoms of secondary syphilis are syphilitic exanthem, generalized lymph node swelling and the presence of condylomata lata and mucous patches. Patients often develop general symptoms such as malaise, fever, headache, sore throat, hoarseness, arthralgia and osteodynia. The skin lesions in secondary syphilis can assume a multitude of forms. As a rule the first lesions are the so-called macular syphilids: faintly rose-coloured spots with a diameter of about 1 cm which can appear anywhere on the body but show a predilection for the handpalms and footsoles. After some time these spots, not readily identifiable and therefore sometimes overlooked by the patient, turn into papules with the same generalized distribution; later, desquamation on the papules may give rise to papulosquamous or (especially on handpalms and footsoles) psoriatiform efflorescences. Especially on the skin of the face and scalp, pustules may form which sometimes impress as acneiform, while in other cases more sharply elevated papulo-pustules are seen in follicular arrangement. Patchy loss of hair is another very typical feature: ill-defined patches show unmistakably less hairgrowth, and differentiation from alopecia areata is therefore not difficult. This syphilitic alopecia is known in Anglo-American literature as 'moth-eaten alopecia'. Condylomata lata are characteristic efflorescences at the transition between skin and mucosa. They are flat, hypertrophic, flesh-coloured papules with a broad base and a greyish-white or eroded surface. They can also occur at other warm, moist sites, e.g. scrotum, axillae, umbilicus and between the toes. The most frequent localizations are the perianal area, genitals and corners of the mouth. Mucous patches can also occur: slightly elevated oval plaques

with a greyish -white surface on the genitals, anal mucosa and in the oral cavity. Owing to the very superficial presence of treponemata, condylomata and mucous patches are the most infectious lesions in secondary syphilis. Other, more general symptoms in secondary syphilis can be: acute uveitis, hepatomegaly and splenomegaly as an expression of hepatitis, sometimes associated with jaundice and headache, as an expression of meningeal irritation. When left untreated, the efflorescences can disappear after 1-3 months. Some 25% of these patients subsequently show relapse or exacerbation of the skin lesions.

2.6.2.3. Diagnosis

As in primary syphilis, dark-field microscopy is necessary also when secondary syphilis is suspected. It can be done without difficulty by obtaining specimens from the eroded surface of condylomata lata or mucous patches, or after scarification of a papule on the intact skin. In addition, serological tests (both treponemal and non-treponemal) are positive in 100% of cases, with high titres in the non-treponemal tests. Histological examination can also contribute to diagnosis, particularly in cases with atypical symptoms (Notowicz et al., 1975).

2.6.2.4. Differential diagnosis

The already mentioned variety of dermatological symptoms in secondary syphilis of course implies the necessity of differentiation from a large number of dermatoses. To be taken into account in particular are other causes of generalized exanthem (e.g. toxicoderma, virus diseases) and erythemato-squamous conditions (e.g. pityriasis rosea, seborrhoeic eczema, psoriasis) and conditions characterized by the presence of papules (e.g. lichen ruber).

2.7. Early and late latent syphilis

After weeks or months, secondary syphilis can show gradual transition to the latent phase, characterized by definition by the absence of clinical symptoms. The patient harbours T. pallidum in the lymphoid tissue (Collart et al., 1964), and there seems to be a state of equilibrium between parasite and host. In the latent phase, the diagnosis is established on the basis of repeated unchanged positive serological tests in the absence of clinical symptoms and without indications of neurosyphilis and cardiovascular syphilis. Further investigation by the organ specialists involved is

required for this purpose. Latent syphilis can be divided into early and late latent forms, dependent on the interval since infection. A condition is diagnosed as early latent syphilis when anamnestic and/or epidemiological data suggest that the infection is of less than 2 years' standing and when treponemal serological tests are positive in the absence of clinical symptoms. Lymphadenopathy may be the sole manifestation. Early latent syphilis is a stage of early infectious syphilis. In the case of an infection of more than 2 years' standing, the condition is diagnosed as late latent syphilis.

In some cases with positive serological tests without clinical symptoms and with no anamnestic evidence of syphilis, differentiation from yaws or other treponematoses is necessary, particularly when the patients come from regions where these diseases are still endemic. In The Netherlands, this has to be taken into account in particular in the interpretation of positive serological tests in patients who originate from Surinam (Menke et al., 1979; Niemel et al., 1979). In some instances it may be necessary also to differentiate between acquired latent syphilis and latent congenital syphilis.

The importance of the detection and treatment of cases of latent syphilis lies in the fact that this can prevent development of tertiary syphilis in the majority of cases.

2.8. Late syphilis (tertiary syphilis)

Studies by Gjestland (1955) concerning a group of untreated syphilis patients collected by Boeck during the period 1891–1910, show that about 33% of the untreated patients recover spontaneously, while another 33% remain in the latent stage for life, and a final 33% develop signs of late (tertiary) syphilis. Symptoms of late syphilis can develop 3–12 years after infection, but often occur even much later. In this stage, dark-field microscopy of specimens from the lesions is useless because only few treponemata are present in this material. The non-treponemal serological tests are often negative, but may be strongly positive in active late symptomatic syphilis. Treponemal syphilis tests are nearly always positive. The incidence of late symptomatic syphilis has substantially decreased as a result of the penicillin treatment of early syphilis.

Of the various forms of symptomatic syphilis, only gummatous syphilis, cardiovascular syphilis and neurosyphilis will be briefly described.

It should always be borne in mind that, as a result of the bacteraemia in

the early stage of the disease, treponemata can have settled anywhere and in any organ system.

2.8.1. Gummatous syphilis

A gumma should be regarded as a violent, mainly cellular reaction to the presence of a small number of treponemata with which the organism has previously co-existed in peace. A solitary gumma or multiple gummata may be present. A gumma is histologically characterized by localized or diffuse chronic granulomatous inflammation. In the localized form, one observes a central necrotic area surrounded by granulation tissue and a more peripheral zone of fibrosis. The granulation tissue contains a perivascular infiltrate of lymphocytes, plasma cells, epitheloid cells and giant cells. The surrounding tissue shows endarteritis obliterans. Gummata can be found in skin, subcutaneous tissue, mucosa, bones and visceral organs. Nodose or tuberculo-ulcerous lesions in tertiary syphilis of the skin are the most superficial forms. Like the gummatous forms, they are solitary, asymmetrical, indurated and painless; they often show a sharply defined arched boundary, and may be associated with central necrosis with a tendency to central or unilateral healing with cicatrization. Polycyclic lesions with irregular contours can thus develop. In some cases the lesions can be covered by psoriasiform squamae. These lesions (like those in tuberculosis of the skin, for example) can show an applesauce colour at diascopy. Gummata are as a rule localized at a deeper level, in the subcutis, and can spread both to the surface and to deeper levels. The absence of pain is a striking feature. As a rule there is central ulceration with perforation to the outside or into one of the body's cavities. In that case there results a typical punched-out ulcer with necrotic margins, and often also a polycyclic demarcation due to perforation at several sites. Localizations of predilection are the face, scalp, chest and calves. Gummata in the tongue can occur in either solitary or multiple form, sometimes with ulceration, and sometimes with diffuse infiltration producing the features of chronic interstitial glossitis, which should be regarded as precarcinomatous. Destruction of the nose produces a characteristic feature of late congenital syphilis: the so-called saddle-back nose which results from collapse of the nasal septum.

2.8.1.1. Differential diagnosis

All conditions associated with granuloma formation and/or ulceration are to be considered: tuberculosis, leprosy, sarcoidosis, chronic pyoderma, iododerma and tumours.

2.8.2. Cardiovascular syphilis

Vascular lesions are very important in the pathogenesis of organ lesions in syphilis. Clinical symptoms of cardiovascular syphilis usually develop late: 10–30 years after the infection. Some form of neurosyphilis is present in 25% of the patients with cardiovascular syphilis.

2.8.2.1. Clinical symptoms

The principal symptoms in the majority of cases are symptoms of aortitis as a manifestation of inflammatory lesions in the aortic wall. Endarteritis of the vasa vasorum leads to ischaemic changes, followed by degeneration of the elastic tissue of the tunica media of the vascular wall, and fibrosis. The intima of the aortic wall is likewise damaged and shows characteristic cicatrization with atheroma formation and calcifications. This damage to the aortic wall may give rise to aneurysmal dilatations, which sometimes produce characteristic changes in the chest X-ray. The ascending aorta is most frequently affected, with lesions beginning close to the aortic valves so that aortic insufficiency may develop. The coronary ostia may also be affected, with clinical symptoms of coronary ischaemia. True syphilitic myocarditis and gummata in the aortic wall or myocardium are rare; given a cardiac localization, they may give rise to arrhythmia. Aneurysms of the aortic arch, descending aorta and abdominal aorta can also occur, but are very rare.

2.8.3. Neurosyphilis

Invasion of the central nervous system and meninges occurs in early syphilis during generalization of the infection. In the majority of cases this causes no symptoms; headache and signs of mild meningeal irritation can as such be part of the symptomatology of secondary syphilis. Acute syphilitic meningitis is a rare development in early syphilis.

2.8.3.1. Diagnostic procedures

Apart from a detailed history, clinical neurological examination and serological blood testing, cerebrospinal fluid studies are of essential

importance. Of the various laboratory procedures – e.g. cell count, total protein, globulin, serological tests and colloid tests – the first two in particular are very sensitive but give no specific criteria.

2.8.3.2. Asymptomatic neurosyphilis

The term asymptomatic neurosyphilis applies when there are cerebrospinal fluid changes of a pattern consistent with neurosyphilis, but no clinical neurological symptoms, in a patient with latent syphilis, treated or untreated. This condition certainly requires treatment and, when left untreated, can progress to symptomatic neurosyphilis (Van Vliet, 1973).

2.8.3.3. Late signs of symptomatic syphilis of the nervous system

The late signs of syphilis of the nervous system can be divided into two main forms:

a. Meningovascular syphilis
The blood vessels of the brain, spinal cord and meninges show proliferative endarteritis with a perivascular infiltrate of lymphocytes and plasma cells, followed by fibrosis and tissue necrosis due to thrombotic processes. The symptomatology usually varies widely with the different localizations of the process within the central nervous system. The principal early symptoms of this form of neurosyphilis are irregular, unequal pupils with a delayed response to light (Argyll Robertson pupils) and abnormal leg reflexes. Cerebral signs of meningovascular syphilis are basal gummatous meningitis, cerebral endarteritis and, rarely, cerebral gummata. Meningomyelitis, syphilitic amyotrophy, spastic paraplegia, hypertrophic pachymeningitis and spinal endarteritis are among the spinal signs of this form of neurosyphilis.

b. Parenchymal syphilis
This form is subdivided into:
1) Dementia paralytica:
In dementia paralytica there is involvement of the cerebral parenchyma with atrophy of the cerebral cortex. The earliest symptoms are generally intellectual deterioration, loss of memory, reduced ability to concentrate, faulty judgement and changes of mood, later followed by delusions and dementia. Neurological changes include tremors, pupillary abnormalities, dysarthria, dyslexia, convulsions, incontinence and pyramidal symptoms.

2) Tabes dorsalis:

Tabes dorsalis involves the spinal cord. Characteristic features are ataxia of the lower limbs and signs of degeneration of the dorsal columns. The clinical symptomatology varies widely and includes fulgurant ('shooting') pains in the legs, ataxia, numbness, disturbed bladder function, impotence, diminished visual acuity, severe abdominal pains and arthropathies. Associated neurological changes are very extensive.

2.9. Pregnancy and syphilis

2.9.1. Introduction

The diagnosis of syphilis in pregnant patients does not differ in principle from that of syphilis in non-pregnant patients. However, the distinction between early infectious syphilis and late latent syphilis is very important with a view to the timing of treatment. Serological tests for syphilis are routinely performed in every pregnant woman. Since false-positive non-treponemal tests are occasionally seen during pregnancy, it is important to perform at least one treponemal serological test. Another important argument in favour of this is the fact that titres of non-treponemal antibodies tend to fall with a longer duration of syphilis (and can totally disappear after some time). This explains that non-treponemal tests can be negative in a pregnant woman even though she is suffering from late syphilis - as demonstrated by a positive treponemal test.

2.9.2. Mode of infection

Intrauterine infection takes place via placental passage of treponemata, which is not possible until after the fourth month of pregnancy. It is therefore useful to test the pregnant woman for syphilis in the earlier phase of pregnancy (e.g. the second or third month). Early diagnosis allows early institution of treatment, i.e. before the fourth month. Since a pregnant woman can still be infected with syphilis after the third month, theoretical it is advisable to repeat syphilis tests towards the end of term.

2.9.3. Therapeutic approach of the pregnant patient

The treatment of pregnant syphilis patients primarily aims at prevention of congenital syphilis in the neonate to be. Women with early syphilis (primary, secondary, early latent) should be treated as soon as the diagnosis is established. In late latent syphilis, the maximum preventive effect of treatment cannot be expected until the fourth-to-fifth month of pregnancy.

Treatment later in the course of pregnancy is intended as curative treatment of a possibly already infected foetus. Whether treatment should be repeated during a subsequent pregnancy is considered by some authors to depend on the outcome of the Treponema Pallidum Immobilization (TPI) test. As long as the TPI test remains positive, T. pallidum may be present in the patient's body (Collart, 1962). The presence of T. pallidum constitutes a potential danger of infection of the foetus, and thus provides an argument in favour of repeated treatment during a subsequent pregnancy (Degos, 1971). Other authors consider the risk of a neonate with congenital syphilis to be much smaller after adequate treatment during a previous pregnancy, and therefore advise against repeated treatment during a subsequent pregnancy (Lindemayr and Partsch, 1976).

2.9.4. Conduct at delivery and during the first months of life of an infant with possible congenital syphilis

Women in whom early syphilis has been diagnosed, should be delivered in a clinical setting. Whether this is also necessary in the case of late latent syphilis, is debatable. In any case it is important to submit every neonate with a syphilitic mother to a detailed physical examination for evidence of congenital syphilis, regardless of the stage of the mother's disease and the nature of the treatment given.

Serological tests are very important in diagnosing neonatal syphilis. Positive tests in the neonate can be based either on passive transfer of antibodies from mother to child, in which case the latter is not infected, or on active production of antibodies in the child, in which case the child is infected. If at the time of delivery the neonatal antibody-titres are higher than those in the mother, infection of the neonate is a possibility to be seriously considered. A fall of the titres during the first three month after birth should be regarded as an argument against infection, whereas unchanged or rising titres provide an argument in favour of infection. In actual practice the diagnosis of congenital syphilis may be problematic, and some time may pass before the diagnosis is established. The possibility to perform the FTA-IgM test can be an asset in the diagnosis of congenital syphilis, because IgM antibodies specifically indicate a recent infection.

2.9.5. Consequences of maternal syphilis for the foetus

Syphilis during pregnancy can have the following consequences for the foetus:

1) premature birth (less than 16 weeks of pregnancy);
2) intrauterine death late in the course of pregnancy;
3) birth with signs of syphilis;
4) birth in apparent health, but development of signs of early congenital syphilis after a few weeks or months;
5) late congenital syphilis (symptoms developing after the second year of life);
6) no infection.

2.9.6. Diagnosis of syphilis in neonates

Three points are important in the diagnosis of syphilis in neonates:

1) Examination of the placenta: in the case of syphilis this is too heavy and pale, and shows typical histological changes (endarteritis and an infiltrate of mostly plasma cells).
2) Examination of the neonate (with special reference to symptoms of early congenital syphilis).
3) Serological testing.

2.9.7. Early congenital syphilis (infectious, 0–2 years)

Early congenital syphilis is rare in The Netherlands. Symptoms may be marasmus, senile face, yellowish-brown (café-au-lait) complexion, snuffles. The skin may show macular, papular, maculopapular, bullous and polymorphous exanthems of symmetrical localization, especially around the mouth, in the anogenital region and on the handpalms and footsoles. Additional symptoms include onychia, paronychia, condylomata lata and osteopathies such as osteochondritis (epiphysitis), Parrot's pseudo-paralysis and periostitis. There may also be hepatomegaly associated with syphilitic cirrhosis, splenomegaly, pneumonia, syphilitic meningitis, asymptomatic neurosyphilis, iritis and chorioretinitis.

2.9.8. Late congenital syphilis (non-infectious, age over 2)

Late congenital syphilis is likewise rare in The Netherlands, but less rare than early congenital syphilis. The principal symptoms are gummata, preferable in the nose and mouth, with subsequent malformation of the face. There is also periostitis, often of the tibiae and leading to deformity

(sabre-shaped tibiae). Interstitial keratitis, chorioretinitis, uveitis and optic atrophy are important ophthalmological findings. Synovitis with painless swelling and hydrops (so-called Clutton joints) produces characteristic radiological changes. Physical and mental retardation and clinically manifest neurosyphilis (juvenile tabes dorsalis and dementia paralytica) were in the past interpreted as evidence of 'constitutional inferiority' of these patients.

2.9.8.1. Stigmata of late congenital syphilis

Stigmata are marks which the patient carries all his life as evidence of his (her) intrauterine infection with syphilis. Scars and deformities resulting from some of the abovementioned conditions are often permanent. Localized thickening due to periostitis of the frontal and parietal cranial bones results in a skull of characteristic appearance. Malformation of the nasal bones leads to flattening of the dorsum of the nose: the so-called saddle-back nose. The palate is often arched high. The mandible protrudes and causes a bulldog face. The teeth likewise show typical abnormalities (Hutchinson teeth). Other possible stigmata are: corneal cicatrization, perforation of the nasal septum or palate, sabre-shaped tibiae, long-standing choroiditis, optic atrophy and sensorineural hearing loss.

2.10. Therapy

Penicillin, which acts on the dividing treponema, is the agent of choice in the treatment of syphilis. The general principle is that a penicillin concentration of at least 0.03 U/ml serum should be maintained during several times the generation period of T. pallidum (about 33 hours). Whether this generation period of about 33 hours also applies to T. pallidum in the human organism during a longstanding syphilis infection, is not entirely certain. For lack of an in-vitro cultivation technique, there are no exact studies of the sensitivity of T. pallidum to antibiotics. Although penicillin has been used in the treatment of syphilis for over 30 years, there seems to be no diminished penicillin sensitivity of the treponemata. T. pallidum-like structures have been repeatedly demonstrated in the eyes, cerebrospinal fluid, lymph nodes and bones of patients formerly treated for early or late syphilis (Editorial 1968). In some of these cases it was demonstrated by inoculation of rabbits that these structures were indeed virulent T. pallidum. The consequences of their persistent presence (can they cause a flare-up of symptoms; can they infect the foetus in utero?)

are not clear. The question arises whether the generally accepted minimum treponemicidal penicillin concentration and the presumable generation period of T. pallidum are in fact correct.

A great many syphilis therapies have been described, which can be grossly divided into two categories: the relatively short Anglo-American and the relatively long European penicillin courses. The arguments in favour of the longer courses are based on the above-mentioned uncertainties about the generation period of T. pallidum and the apparent possibility of persistence of the causing organism in spite of therapy.

In several Dutch university hospitals, early syphilis and late latent syphilis without organ lesions were treated by fairly long courses of 9 - 10.8 million IU procaine-penicillin in oil with 2% aluminium monostearate (Almopen®, PAM) intramuscularly, divided over a period of 4-6 weeks. Almopen has been unavailable in The Netherlands since May 1980. The Rotterdam university hospital now uses aqueous procaine-penicillin G (Depocillin®) - 600.000 IU daily intramuscularly over a period of 2-3 weeks.

The guidelines of the American Ministry of Health (Center for Disease Control, Atlanta) indicate substantially shorter courses of treatment: for early syphilis, 2.4 million IU benzathine penicillin (Penidural®) intramuscularly or 4.8 million IU aqueous procaine-penicillin G over a period of 8 days, or procaine-penicillin in oil with 2% aluminium monostearate - 2.4 million IU to begin with, followed by 2 x 1.2 million IU at three-days intervals.

In late latent syphilis in which no further examination has been done, or in verified symptomatic syphilis, more intensive therapies are indicated e.g. 1.2 million IU procaine-benzylpenicillin/sodium-benzyl-penicillin (3:1) (Bicilline®) daily for two weeks, followed by 1.2 million IU benzathine penicillin/procaine-penicillin G/potassium-penicillin G (2:1:1) (Penidural DF®) twice weekly during four weeks, up to a total of 31,2 million IU penicillin. In early and late congenital syphilis (up to 12 years) treatment with 20.000 U Bicilline® per kg body weight during six weeks is recommended.

In a recent investigation it was shown that, in the treatment of neurosyphilis with 1.2 million IU benzathine penicillin thrice weekly or with a daily intramuscular dose of 600.000 IU procaine-penicillin G, no treponemicidal concentrations in the cerebrospinal fluid were attained (Dunlop et al., 1979). It was however possible to obtain such concentrations by

intramuscular injection of 4 x 500.000 IU aqueous penicillin G in combination with 500 mg probenecid by mouth, or by daily intramuscular injection of 2.4 million IU aqueous procaine-penicillin G in combination with 4 x 500 mg probenecid per day by mouth. Alternative courses of antimicrobial therapy are conducted with tetracycline hydrochloride or erythromycin, 2 g per day by mouth in four divided doses during 30 days.
Views on follow-up also differ widely. In any case a serological follow-up should take place at regular intervals. In addition a follow-up with special reference to possible late symptomatic syphilis should be made in collaboration with the relevant organ specialists 2 years after early syphilis.

2.10.1. Side effects of treatment with penicillin

Treatment of syphilis with penicillin can give rise, not only to various allergic reactions but also to two unusual side effects. The first of these is an acute non-allergic reaction after administration of aqueous procaine-penicillin (Hoigné's syndrome). This syndrom in its typical form is characterized by severe acute sense of depersonalization, verbalized fear of death and hallucinations. On examination these patients are shown to have tachycardia, increased blood pressure and dilatation of the pupils. These symptoms are quickly reverted by intramuscular administration of 10 mg diazepam (Pepplinkhuizen et al., 1977). The second side effect is the Jarisch-Herxheimer reaction, either systemic with fever and leucocytosis or localized with exacerbation of existing skin lesions. There are several aetiological theories. The reaction might be due to release of endotoxins from destroyed treponemata, or release of leucocytic pyrogens after phagocytosis of membrane fragments from treponemata. Release of histamine as a result of degranulation of mast cells and basophils in response to combination of treponemal antigens with membrane-bound antitreponemal IgE might also explain some features of this reaction (Bos et al., 1980a). This reaction generally requires no therapy, but the patient should be warned that it may occur.

CHAPTER 3

SEROLOGICAL TESTS IN SYPHILIS. A GENERAL SURVEY

3.1. Introduction

Serological tests used to detect and identify syphilis are divided into non-treponemal and treponemal tests, on the basis of the antigen used. Non-treponemal or lipoid tests involve the use of a non-specific antigen: cardiolipin, in combination with lecithin and cholesterol. Treponemal tests involve the use of antigens directly derived from treponemata or of the entire intact treponema. According to origin, and therefore according to specificity in regard to T. pallidum, the tests in this group are further divided into group-specific and type-specific treponemal tests.
This division will be used in a survey of various tests currently performed in The Netherlands, with special reference also to a few new modifications not yet introduced here. Two concepts of importance in interpreting results of serological tests will also be discussed: anti-complementarity and biologically false-positive tests.

3.2. Non-treponemal tests

Antibodies demonstrable with the aid of non-treponemal tests used to be known as reagins*. These antibodies develop after infection with T. pallidum, but can also be found in other diseases and during pregnancy. They are able to unite with suspensions of lipid extracts of animal and vegetable origin, aggregating to form visible masses of the kind observed in flocculation tests. These masses are able to unite with complement, and this ability underlies the use of the complement fixation test.

3.2.1. Complement fixation tests

Wasserman et al. (1906) were the first to describe the use of a complement fixation test with, as antigen, an aqueous extract of livers of foetuses deceased from congenital syphilis, in the serological diagnosis of syphilis. There followed a long series of modifications, the most important of which were the use of aqueous or alcoholic extracts of normal organs as antigen,

---

* The term reagin is currently confined to the IgE-class antibodies in atopy, and will not be further used here in order to avoid confusion.

and addition of cholesterol and lecithin to enhance the sensitivity of the test. The procedure described by Kolmer (1922, 1948) is still being used as the Kolmer complement fixation test in The Netherlands. This test is inexpensive, can be quantitatively performed and permits a high degree of automation.

3.2.1.1. Anti-complementarity

The term anti-complementarity applies when no haemolysis occurs in the control tube of a complement fixation test, i.e. without antigen but in the presence of the patient's serum. This phenomenon is often ascribed to complement-consuming complexes made up of cryoglobulins, rheumatoid factors or large amounts of γ-globulins. The repeated occurrence of anti-complementary serological tests can be indicative of an immunological abnormality or affection of the liver (Lassus and Mustakallio, 1973), and therefore always calls for further internal examination (Schuller, 1973; Van de Merwe, 1975).

3.2.2. Flocculation tests

E. Meinicke (1917) was the first to describe a practicable flocculation test for syphilis, with the aid of the same antigen as used in the complement fixation test. Numerous variants of this flocculation test have been described in the course of the years. They included the VDRL (Venereal Disease Research Laboratories) test, which is still widely used in The Netherlands (Harris, 1946). This test, usually performed as a micro-flocculation test, can be automated to a large extent. Its advantages in comparison with the Kolmer test are rapidity of performance and independence of a haemolytic system. In 1962, Portnoy et al. described the RPR (Rapid Plasma Reagin) card test, performed with the aid of a stable VDRL antigen suspension which contains carbon particles and which, with positive serum, causes flocculation which is readily made macroscopically visible by the carbon particles. An important advantage of this test over the classical VDRL test is simplicity of procedure and availability of a distinct and readily readable result within 10 minutes. The RPR card test is used in The Netherlands on a limited scale at a few major out-patient clinics, particularly as quick screening test. The test can also be performed quantitatively. In recent years, modifications of flocculation tests have been developed with special emphasis on automation (ART, Automated Reagin Test) and on a more clear readability of the test result. These are

the Reagin Screen Test (RST), which makes use of a fat-soluble blue dye, and the Syphla-Chek test in which the antigen, conjugated to kaolin and choline chloride, gives rise to the formation of light-coloured granules at flocculation. In comparison with the RPR card test, the advantages of these tests are not evident (Black et al., 1976; Dzink et al., 1977).

3.2.3. Biologically false-positive tests

In a number of cases, non-treponemal tests give a positive result in patients in whom a treponemal infection can be excluded on the basis of the medical history, clinical findings and epidemiological data. Serum from a person not suffering from treponematosis can contain minute amounts of non-treponemal antibodies (Kahn and Malloy, 1931) that are not demonstrable with the aid of the conventional serological tests. During an infection with some other microorganism, this amount can increase sufficiently to give rise to a positive non-treponemal test, usually in low titres. This can also occur in auto-immune diseases, after vaccination, during pregnancy and in heroin addicts. These so-called biologically false-positive tests are further divided, according to the duration of their presence, into acute (maximally 6 months) and chronic (exceeding 6 months) biologically false-positive tests. In view of the fact that fairly serious diseases are occasionally found in patients with chronic biologically false-positive tests (Johannson, 1970; Catterall, 1972; Schuller, 1973), further internal examination of these patients is advisable.

3.3. Treponemal tests

As the limitations imposed on the classical serological tests by the antigen used gradually emerged, the need for tests using treponema-specific antigens became more urgent.

3.3.1. Group-specific treponemal tests

3.3.1.1. Reiter protein complement fixation test (RPCF test)

Efforts were made to use the non-virulent Reiter treponema, cultivated in vitro, as antigen.* Gaehtgens (1929) already reported good results obtained with phenolated Reiter treponema suspensions. It was subse-

---

* On the basis of comparative studies of the antigenic structure of non-pathogenic treponemata, Reiter's treponema is now classified as T. phagedenis, biotype Reiter.

quently demonstrated that this antigen contains only a lipid component, which is also responsible for the classical non-treponemal tests (Eagle and Hogan, 1940). In an attempt to improve the specificity of antigens from Reiter treponema, d'Alessandro et al. (1950) concerned themselves in particular with the antigenic structure of T. reiteri. They isolated a protein fraction against which antibodies were demonstrable in syphilis patients. This protein antigen proved to react with serum from patients with syphilis and other treponematoses, and with Reiter antiserum. It was therefore assumed to be a group-specific antigen. A group-specific antigen is defined as an antigen which reacts with antibodies against several treponema species, i.e. against the causative agents of syphilis and other treponematoses as well as non-pathogenic treponemata. This antigen is prepared by precipitating an ultrasonicate of Reiter treponemata with 70% saturated ammonium sulphate. It is used in a complement fixation test now known as Reiter Protein Complement Fixation (RPCF) test. This test is still in use at many laboratories and permits a high degree of automation. A disadvantage of the RPCF test is that it sometimes produces solitary weak-positive results in the absence of any other indications of an infection with pathogenic treponemata. It must be assumed that a cross-reaction with antigens from non-pathogenic treponemata is involved in these cases. Moreover, the occurrence of anti-complementary tests can pose a problem.

3.3.1.2. Reiter protein counter-immuno-electrophoresis (RPCIE) test

Bänffer et al. (1974, 1975) used the Reiter antigen in a counter-immuno-electrophoresis technique. As advantages they mentioned a higher specificity than the RPCF test and independence of a haemolytic system, thus eliminating possible anti-complementarity.

3.3.2. Type-specific treponemal tests

3.3.2.1. Treponema pallidum immobilization (TPI) test

In the context of an otherwise unsuccessful attempt to evolve a synthetic nutrient medium for T. pallidum, Nelson and Steinman (1948) succeeded in obtaining a liquid medium in which T. pallidum could survive a number of days without multiplying. It was found that serum from syphilis patients can contain specific antibodies which, together with complement, are able to immobilize virulent T. pallidum (Nelson and Mayer, 1949). This immobilization is microscopically verified. The principle of this Treponema

Pallidum Immobilization (TPI) test is simple: virulent T. pallidum micro-organisms - obtained from inoculated rabbit testes - are mixed with patient's serum and complement. After 18 hours' incubation, the immobilization of the treponemata is microscopically assessed and quantified. However, the test is technically complicated and time-consuming, and consequently expensive. It is used at only a limited number of laboratories in the world (in The Netherlands: the National Institute of Public Health, Bilthoven). The specificity of this test proved to exceed that of all other tests then available, even though it soon became apparent that even with this test it was not possible to differentiate between syphilis and conditions caused by other treponemata, e. g. yaws and pinta.
Subsequently, numerous efforts have been made to demonstrate treponemal antibodies by other techniques and other tests. Treponemal antigens were again used, more specifically dead pathogenic or non-pathogenic treponemata. Due to problems with the production and standardization of stable antigens, most of these tests - e. g. Treponema Pallidum Agglutination (Magnuson and McLeod, 1956), Treponema Pallidum Immune Adherence (Olansky et al., 1954) and Treponema Pallidum Complement Fixation (Portnoy and Magnuson, 1956) - have not been further developed.

3. 3. 2. 2. Fluorescent treponemal antibody absorption (FTA-ABS) test

Deacon, Falcone and Harris in 1957 described a test in which a fluorescence technique was used to demonstrate antibodies. This Fluorescent Treponemal Antibody (FTA) test made use of intact killed T. pallidum, dried on a microscope slide and incubated with a serum dilution (initially 1:5 but later, in view of many false-positive tests, increased to 1:200). After washing, a fluorescein-conjugated anti-human γ-globulin is added, and eluted again after incubation. In UV light the treponemata then show a bright green fluorescence - at least when antibodies from the patient's serum are bound. Hunter et al. (1964) introduced initial absorption of the test serum with intact Reiter treponemata. This was based on earlier studies (Deacon and Hunter, 1962) which had shown that Reiter treponemata have certain antigenic determinants in common with T. pallidum. After absorption the specificity of the test (now called FTA-ABS test) was substantially increased without affecting its sensitivity. Later, a different absorbent was used for this purpose: the so-called sorbens, which is a concentrate of the supernatant of a heat-killed culture of Reiter treponemata (Stout et al., 1967). This test soon proved to be very

sensitive both in untreated and in treated syphilis; in fact its sensitivity exceeded that of all other tests then available. Patients with rheumatoid factor or other auto-antibodies in the organism occasionally show a positive FTA-ABS test in the absence of any indication of a treponemal infection. Patients with lupus erythematosus likewise often show positive fluorescence in the FTA-ABS test; this has a characteristic beaded pattern which clearly differs from the homogeneous fluorescence usually observed in this test. False-positive FTA-ABS tests have also been described in certain other conditions, like diabetes mellitus (Hughes et al., 1970), pregnancy (Buchanan et al., 1970) and herpes genitalis (Wright et al., 1975). Feeble, not readily interpretable fluorescence patterns are not infrequently observed in otherwise quite healthy persons; they may or may not be reproducible (Burns, 1975; Dans et al., 1977).

3.3.2.3. Fluorescent treponemal antibody absorption IgM (FTA-ABS-IgM) test

Scotti and Logan (1968) were the first to describe application of the FTA-ABS test with a mono-specific conjugate, focused solely on demonstration of IgM antibodies. This test proved to be positive in neonates with congenital syphilis, but negative in unaffected infants of mothers with positive syphilis tests. The thus demonstrated presence of specific IgM-class antibodies might be indicative of active antibody production in the infant, in response to an infection with T. pallidum. The sensitivity of the test is about 90% in early symptomatic congenital syphilis, but much lower in the most common type of congenital syphilis, with later development of clinical symptoms: 65%, i.e. lower than the sensitivity of the routine FTA-ABS and VDRL test (Kaufman et al., 1974). It was found with the aid of this FTA-ABS-IgM test that specific antitreponemal IgM can be present, not only in early syphilis but also in untreated latent and late forms of syphilis.

False-positive results may be found in the presence of rheumatoid factor (IgM anti-IgG antibodies) in the serum (Wilkinson and Rodin, 1976; Shannon and Booth, 1977). Rheumatoid factor can be present in the serum of syphilitic adults (Mustakallio et al., 1967; Perrot et al., 1971), but also in the serum of neonates with congenital infections. According to Reimer et al. (1975), syphilitic neonates produce insufficient amounts of IgM antibodies against T. pallidum, but instead produce relatively large amounts of IgM anti-IgG antibodies. The presence of maternal IgM in the

foetal circulation as a result of materno-foetal transfusion, can also cause false-positive results. Pollution of the FTA-ABS-IgM reagent with IgG can likewise influence the test result (Luger et al., 1977). Blockage of the IgM determinants on the antigen surface by excessive amounts of IgG antibodies can cause false-negative results (Wilkinson, 1976; Müller, 1977). Complete separation of IgM and IgG antibodies followed by an FTA-ABS-IgM test with only the 19S-IgM serum fraction substantially enhances the test specificity but for practical reasons cannot be used as routine technique (Schmidt, 1979). Both positive and negative results of the FTA-ABS-IgM test should be interpreted with caution, and the practical value of this test is limited for the time being.

3.3.2.4. Treponema pallidum haemagglutination assay (TPHA)

Rathlev (1965) and Tomizawa and Kasamatsu (1966) almost simultaneously described the use of a passive haemagglutination technique in demonstrating antibodies against T. pallidum. The technique involves the use of tannin-treated sheep erythrocytes, sensitized with an ultrasonicate of T. pallidum. The first commercially available version from Japan uses sheep erythrocytes. In this test an absorption procedure is carried out with the intention of eliminating possible cross-reacting antibodies against sheep erythrocytes and non-pathogenic treponemata. Otherwise these cross-reacting antibodies might influence the specificity of the test. Sequeira and Eldridge (1973) used turkey erythrocytes for the haemagglutination, without preceding absorption. The advantage of this test is that haemagglutination is visible within 1 hour of addition of the test serum if it contains antibodies (whereas with sheep erythrocytes this takes 4 hours). The TPHA can be easily performed as micro-method, and can also be largely automated. The test was soon found to be more sensitive than the non-treponemal tests. Its important advantage over the FTA-ABS test is easy readability without special aids. Its sensitivity seems to be inferior to that of the FTA-ABS test in primary syphilis, but may be superior to it in late and treated syphilis. The test often remains positive even after treatment (perhaps more frequently than the FTA-ABS test). Treatment has no evident effect on test titres.

Views on the specificity of this test differ. Blum et al. (1973) found false-positive TPHA results in a large number of patients, and Ovcinnikov (1974) observed many false-positive TPHA results in patients with cancer and with various dermatoses. Lesinsky et al. (1974) concluded that the

test specificity, although high in a general population, is substantially less than that of the TPI and FTA-ABS tests in patients known to have produced biologically false-positive non-treponemal tests. In view of the results reported by Tomizawa et al. in Japan (1969), by Garner et al. in New Guinea (1973) and by Ghinsberg et al. in Israel (1972), Kiraly and Prerau (1974) maintained that the specificity of the TPHA diminishes substantially when used with sera from patients who come from tropical and subtropical regions. Many investigators in Western Europe (O'Neill et al., 1973; Luger and Spendlingwimmer, 1974; Spendlingwimmer, 1976; Ehrke et al., 1977; Müller, 1977) found the specificity of the TPHA to equal that of the TPI and the FTA-ABS tests. The different views of various investigators on the specificity of the TPHA might be due to the presence of certain non-pathogenic treponema in individuals in the populations studied.

3.3.2.5. Enzyme immuno-assay (EIA)

Veldkamp and Visser (1975) were the first to use the Enzyme Immuno-Assay (EIA) in the serological diagnosis of syphilis. This test involves the use of a T. pallidum ultrasonicate with which one coats the wall of a test tube or the cup of a microtitre plate. After coating the serum is added. With the aid of an anti-human immunoglobulin preparation to which an enzyme is attached, the amount of bound immunoglobulin is determined by measuring the change of colour which occurs after addition of a substrate that is susceptible to the enzyme. An important advantage of this test is that it can be entirely automated, including the reading of results with the aid of a spectrophotometer. Although the first results with this test have seemed favourable, the specificity of the test will have to be further investigated before its routine use can be considered.

CHAPTER 4

AIMS OF THE STUDY

In the course of a microbial infection, different amounts of antibodies can be demonstrated at different times with the aid of serological tests. We know from practice that this is possible also in syphilis. However, the literature supplies no exact data on the course of various serological tests for syphilis, performed within a single well-defined population of patients. In view of this, the following questions were formulated:

1. What is the course of a number of non-treponemal serological tests (Kolmer, VDRL and RPR) and treponemal serological tests (RPCF, FTA-ABS and TPI) when a venereally acquired infection with T. Pallidum is left untreated?

2a. What is the effect of penicillin treatment on the course of the various non-treponemal and treponemal serological tests in patients with venereal syphilis?

2b. Is this course different after two different penicillin treatment courses: 31.2 million IU on the one hand, and on the other hand 10.8 million IU long-acting penicillin distributed over a 6-week period?

In regional public health laboratories in The Netherlands, several different serological syphilis tests are in use. The multitude of tests used, often proves to confuse referring physicians; moreover, increasing use is made of a new test: the TPHA. In view of this, the following questions were formulated:

3a. Can the widely used combinations of serological tests (VDRL/Kolmer and VDRL/RPCF) be considered effective in the detection of syphilis?

3b. To which extent could the increasingly used TPHA contribute to this?

CHAPTER 5

MATERIAL AND METHODS

5.1. Diagnostic criteria

The dermato-venereological department of the University Hospital Rotterdam, applies the criteria formulated by Menke (1975) for the diagnosis of syphilis, and uses the traditional classification into primary, secondary, latent and late symptomatic syphilis.

5.1.1. Primary syphilis

Primary syphilis is diagnosed when a patient shows a localized skin and/or mucosal lesion as first symptom of infection with T. pallidum, alone or in combination with regional lymphadenopathy, and when T. pallidum is demonstrated in the lesion or in a lymph node. On the basis of the results of the following tests – Kolmer, VDRL, RPCF and TPI – patients with primary syphilis are divided into three groups, which correspond with the following stages:

a. Sero-negative primary stage (all serological tests are negative).
b. Sero-changing primary stage (one or more tests are dubious and/or positive, but not all tests are simultaneously positive).
c. Sero-positive primary stage (all tests are positive).

The FTA-ABS and RPR were not always performed at the time of diagnosis of primary syphilis, and therefore are not included in this division.

5.1.2. Secondary syphilis

Secondary syphilis is diagnosed when a patient shows typical, usually generalized lesions of the skin and/or mucosa, alone or in combination with generalized lymphadenopathy. T. pallidum is sometimes demonstrable in material from skin or mucosal efflorescences. Histological findings can support or confirm the diagnosis. Serological syphilis tests are generally strongly positive.

5.1.3. Early latent syphilis

Early latent syphilis is diagnosed when a patient shows no clinical skin or mucosal lesions, and when anamnestic and/or epidemiological data indicate a syphilis infection of no more than 2 years' standing. All serological syphilis tests are usually positive. Lymphadenopathy is often present.

5.1.4. Early infectious syphilis

The term early infectious syphilis encompasses primary, secondary and early latent syphilis.

5.1.5. Late latent syphilis

Late latent syphilis is diagnosed when a patient without clinical lesions shows positive syphilis tests. A positive TPI is the minimum required. It must be plausible on anamnestic and/or epidemiological grounds that the infection is more than 2 years old. Internal and neurological examination reveals no changes consistent with organic syphilis. Congenital syphilis must be excluded on anamnestic and/or epidemiological grounds, supplemented if necessary by the results of an extensive physical examination including ophthalmological and otological examination, and a family study.

5.1.6. Cardiovascular syphilis

This diagnosis is established only on the basis of the findings obtained by an internist or cardiologist.

5.1.7. Symptomatic and asymptomatic neurosyphilis

In patients who have recently or in the past suffered from syphilis, the diagnosis neurosyphilis is made on the basis of characteristic changes in the cerebrospinal fluid (CSF). The following findings or combinations of findings are regarded as characteristic:
Cell count exceeding 7/3, total protein exceeding 50 mg/100 ml, γ-globulin exceeding 20% of total protein, Nonne and/or Pandy test positive, one or several serological syphilis tests positive, characteristic abnormal course of the gold sol curve.
The order of sequence of these findings is also a reflection of the typical pattern of reaction to invasion of the central nervous system by T. pallidum. These findings are not always associated with clinical neurological changes (asymptomatic neurosyphilis). If present, clinical neurological changes can range from slight asymmetry of reflexes to a most extensive symptomatology, as described in Chapter 2.

5.1.8. Endemic treponematosis

Endemic treponematosis is diagnosed in patients with positive serological syphilis tests (at least TPI positive) and clinical signs of one of the endemic treponematoses (yaws, pinta, endemic syphilis), and in patients with a history of endemic treponematosis. In patients from regions where

endemic treponematoses prevail, who show positive serological syphilis tests (TPI positive) but have no history of earlier syphilis or endemic treponematosis, no definite diagnosis is made when signs of symptomatic syphilis are absent. Both late latent syphilis and latent endemic treponematosis are considered 'possible'.

5.1.9. Congenital syphilis

Early and late congenital syphilis are diagnosed on the basis of previously described characteristic findings at general physical examination and dermatological examination, combined with epidemiological data and the results of serological testing (see Chapter 2).

5.1.10. Syphilis - not further classifiable

In a number of cases, insufficient data were available to warrant a definite diagnosis in retrospect. In a number of these cases, however, it could be established with certainty that the patient was suffering from some form of syphilis. These patients were considered to suffer from syphilis - not further classifiable.

5.1.11. Possibly syphilis

Patients in whom one of the above described stages of syphilis was strongly suspected to be present but in whom the diagnosis could not be established with certainty due to absence of some anamnestic and/or epidemiological and/or clinical and/or laboratory data, were regarded as 'possibly suffering from syphilis'.

5.1.12. No syphilis

A number of patients showed a positive result of one or several serological tests (with the exception of the TPI), once or repeatedly, although the presence of syphilis or another treponematosis could be excluded with certainty on the basis of the history, the findings at physical examination, and clinical and epidemiological follow-ups. The records of these patients were marked 'no syphilis'.

5.1.13. Sero-resistance

The diagnosis 'sero-resistance' was made when a syphilis patient, in spite of presumably adequate repeated treatments, failed to show at least a four-fold decrease of the high pretherapeutic titre of non-treponemal antibodies over a follow-up period of at least 2 years.

5.2. Serological testing and laboratory techniques

5.2.1. Serological testing

Blood samples from all patients involved in the study (covering the period from 2nd January 1970 to 30th June 1974) were sent once or several times to the Treponema Division of the National Institute of Public Health in Bilthoven (heads at the time: Dr J. Bekker until 1st January 1973, and Dr J. Veldkamp from 1st January 1973 on) for serological testing. The following serological tests were performed at this institute: Kolmer, VDRL, RPCF, FTA-ABS and TPI (the FTA-ABS test was not performed until after 2nd March 1971). The RPR test was always performed at the routine laboratory for venereal diseases (head: Prof. Dr M. F. Michel) in the dermato-venereological out-patient clinic of the University Hospital Rotterdam. The RPR was intended as quick screening test, mostly upon suspicion of one of the forms of early infectious syphilis, and was therefore carried out in only a limited number of treated patients.

5.2.2. Laboratory techniques

The VDRL flocculation test and the Kolmer complement fixation test were performed by the standard techniques described in the Manual of Tests for Syphilis (1969), with the following modifications.
The VDRL test was performed qualitatively, the result being described by the laboratory as negative, dubious or positive.
The Kolmer test was performed quantitatively, starting with an undiluted series followed by a two-fold dilution series. The results were given as titre, titre being defined as the highest reactive serum dilution, i.e. the first dilution in which less than 50% haemolysis occurs (De Bruyn, 1957).
The RPCF test was likewise performed quantitatively, with the aid of $1\frac{1}{2}$ U complement (De Bruyn and Bekker, 1958). Titration was started with undiluted serum.
The FTA-ABS test was performed as advised in the Manual of tests for Syphilis (1969), with the following modifications: serum dilution was 1:12 instead of 1:5, and the serum was absorbed with an ultrasonicate of *T. reiteri* instead of sorbent. The results were recorded as - (negative), ± (dubious), + (weakly positive), or ++, +++ and ++++ (positive) according to the degree of fluorescence.
The TPI test was performed according to Bekker and Onvlee (1955), with addition of 100 μg lysozyme to the medium (Kent and De Weerdt, 1963).

The results were described as negative, dubious or positive, dependent on the specific immobilization (SI) of treponemata in the test. The specific immobilization was calculated on the basis of the equation:

$$SI = 100 \times \frac{\text{number of live treponemata in control tube minus number of live treponemata in test tube}}{\text{number of live treponemata in control tube}}$$

The TPI test was considered to be positive at SI > 50%, dubious at SI = 20–50%, and negative at SI < 20%. In performing the complement fixation test, a reference serum which had been positive in the Kolmer and RPCF tests was tested along with the test serum samples. This reference serum was supplied by the microbiological reagents division of the National Institute of Public Health, and had been standardized on an international syphilis reference serum from the WHO reference laboratory in Copenhagen. A negative serum was always tested as well. The titre of the reference serum remained constant throughout the study period. A positive and a negative control serum were always included in the qualitative tests VDRL, FTA-ABS and TPI. These sera were positive/negative also in the complement fixation test. The RPR test was performed qualitatively in accordance with the manufacturer's instructions (Hynson, Westcott & Dunning Inc., Baltimore USA); results were recorded as negative (–), weakly positive (+) or positive (++), according to the degree of flocculation.

5.3. Treatment

In the course of the period studied, a number of different treatment plans were applied. During part of the period, for example, treatment of civilians differed from that of sailors. Moreover, dosage and type of penicillin used were changed in the course of the study period.

5.3.1. Civilians

During the period from 2nd January 1970 to 15th December 1972, civilians with sero-positive primary syphilis, secondary syphilis, early latent syphilis, late latent syphilis and late symptomatic syphilis were treated at the dermato-venereological out-patient clinic of the University Hospital Rotterdam. They were given three 6-week penicillin courses with 6-week intervals between the courses. The first course consisted of daily intramuscular injections of 1.2 million IU Bicilline® (procaine-penicillin G and sodium penicillin G 3:1) during the first two weeks, followed by thrice-weekly intramuscular injections of 1.2 million IU Penidural DF® (benzathine

penicillin G, procaine-penicillin G and potassium penicillin G 2:1:1) during four weeks, to a total of 31.2 million IU penicillin. The second and the third penicillin course each consisted of twice-weekly intramuscular injections of 1.2 million IU Penidural DF® during six weeks, to a total of 14.4 million IU penicillin per course. Patients suffering from sero-changing primary syphilis were given two penicillin courses, corresponding with the first and the second course described above. Patients with sero-negative primary syphilis received only the first course. From 15th December 1972 on, the treatment of civilians with primary syphilis (regardless of serological status), secondary syphilis, early latent syphilis and late latent syphilis was changed to a single course of thrice-weekly intramuscular injections of 600.000 IU procaine-penicillin G in oil with 2% aluminium monostearate. (PAM, Almopen®) during six weeks, to a total of 10.8 million IU penicillin.

5.3.2. Sailors

Sailors were often treated in a clinical setting (Havenziekenhuis, Rotterdam; A. P. Djajadiningrat and Dr E. H. Hermans). They received a course which consisted of twice-daily intramuscular injections of 600.000 IU Almopen® during 15 days, with on the day of departure another 2.4 million IU benzathine penicillin G (Penidural®) to a total of 20.4 million IU penicillin. Sailors who could find accommodation in Rotterdam for the duration of treatment, were treated in accordance with the routine then followed at the dermato-venereological out-patient clinic of the University Hospital Rotterdam.

5.3.3. Alternative therapies

Patients for whom penicillin was contraindicated (usually in view of hypersensitivity in the history) were treated with 4 x 500 mg tetracycline hydrochloride daily by mouth during 30 days (total dose 60 g); during the period from 2nd January 1970 to 15th December 1972, one, two or three courses were given on the same indications as penicillin treatment, at 4-week intervals. Subsequently, a single course was considered sufficient. Patients whose penicillin treatment had to be discontinued (usually in view of an allergic reaction) were further treated with tetracycline as described above. Patients who developed hypersensitivity or serious side effects during tetracycline treatment, were further treated with erythromycin by mouth in the same doses as tetracycline. Erythromycin

was also prescribed in these doses when penicillin was contraindicated during pregnancy.

5.4. Methods used in collection, registration and processing of data

The records of all patients first presenting at the dermato-venereological out-patient clinic of the University Hospital Rotterdam, between 2nd January 1970 and 30th June 1974, were systematically examined for deviant findings in each of the serological syphilis tests performed. This implied that data on patients with sero-negative primary syphilis could not be included in the study. In the event of deviant findings, anamnestic, epidemiological, clinical, serological and diagnostic data were recorded on optical readable forms that had been previously used in studies within the dermato-venereological department (Stolz, 1974), and required only slight modifications for the purpose of this study. With the aid of an IBM 1 2 3 4 optical mark page reader linked to an IBM 5 3 4 card-punching unit, the directly readable data were converted to punch-cards (department of system development, Faculty of Medicine, Erasmus University). Further processing was done at the department of automatic signal processing (head: J. Loeve BSc, programmer R. de Haan) of the Central Research Workshops (head: H. A. Bak BSc) of the Medical Faculty, Erasmus University, Rotterdam. After transfer of the punch-card data to a magnetic disc, all further processing was done with the aid of a PDP 11/10 computer. Per patient, data were recorded on three different forms, numbered A1, A5 and L5.

A1 form. Epidemiology and basic data (fig. 5.1):
This form records personal, anamnestic and epidemiological data.
A5 form. Serology (fig. 5.2):
One A5 form was filled out for each date on which serological syphilis tests were performed. A gauge-date system indicated the interval between starting-date and form date in days, weeks, months or years. The results of the serological tests were thus recorded per testing date.
L5 form. Diagnosis and treatment (fig. 5.3):
The diagnosis recorded on the L5 form is the diagnosis established on date 0 (starting-date) on the basis of the criteria listed in section 5.1. Per patient there is one data-set, in which the patient is identified with his case record number and starting-date. The starting-date or date 0 is the first day of treatment or, if no treatment was given, the day on which the first blood sample was obtained.

5.5. Statistical methods

Since it was one of the aims of the study to gain an impression of the chronological course of the results of a given serological test in a group of syphilis patients, an index had to be found to indicate the position of the centre of the laboratory data in a number of patients at a particular time. The arithmetic mean could not be used for this purpose because it is too sensitive to extreme values and therefore would not give an adequate characterization of the series of observations. The median was therefore used. Since grouped observations were involved, the following equation was applied for calculation of the median (Bolle et al., 1973):

$$Me = (X_M - \tfrac{1}{2} h_M) + \frac{\tfrac{1}{2} - F_{M-1}}{f_M} h_M \text{, in which:}$$

Me = median

X_M = mid-point of class M, in which the median lies

h_M = class interval of class M

$X_M - \tfrac{1}{2} h_M$ = the lower bound of class M

F_{M-1} = cumulative relative frequency of class M - 1

f_M = relative frequency of class M.

With this equation for calculation of the median, a measure of the spread of the observations could also be calculated. For this purpose the 25% and 75% value (1st and 3rd quartile) instead of the 50% value of class M was calculated. The equation is consistent also when the consecutive values constitute a geometric progression, as is the case with the reciprocal values of the titres (1, 2, 4, 8, etc.) by taking the logarithm with base 2 from each value. In this way an arithmetic series was obtained which could be used to represent the results of titrated tests in a scale division. The results of tests which the laboratory describes only as negative, dubious, positive or with a number of + signs, were also represented with an arithmetic series. Dubious (±) laboratory results have been regarded as valid laboratory findings lying between negative and positive, or between negative and titer 1:1. In a scale division with gradation according to reactivity of test responses, these were regarded as equidistant (fig. 5.4).

The frequency distribution of the titres of non-treponemal serological tests in syphilis patients during and after treatment is known to be log-normal (Gahlen, 1953; Gahlen and Ninneman, 1965). Logarithmic transformation would make it possible to use tests based on the normal dis-

tribution in the statistical analysis of the results, but nevertheless we applied non-parametric tests. This was done in view of the fact that the type distribution of the treponemal tests FTA-ABS and TPI had not been established with certainty, and also because the possibility of outlying observations made it advisable.
Wilcoxon's two-sample test was used to compare two groups. When the groups were large enough, the normal approximation was used for the test statistic. Wilcoxon's signed-rank test was used to compare observations made within one group on different dates, and the McNemar test was applied to compare different tests within one group on the same date. The level of significance was always 0.05.
The number of observations made per test within one group of patients on one gauge-date was arbitrarily set at a minimum of five in order to be accepted for further processing.

5.6. Graphic representation

An arithmetic scale division according to disease stage is used in Chapter 6 and further explained there. The scale division for the various tests is shown in fig. 5.4. The time scale as used in Chapter 7 is given on the X-axis. A logarithmic scale has been used for the division according to gauge-date (the interval since date 0 being given in days, weeks, months or years). With an arithmetic scale, the X-axis would be impractically long and there would be an unfavourable effect on accuracy (Ferro, 1954). The Y-axis has been divided per test in accordance with the way in which the laboratory reported results. The reciprocal values of the titres are given. All graphs were drawn by a PDP 11/10 computer with Versatec plotter.

5.7. Patients

The total input consisted of data on 1879 patients, recorded on 1879 A1 forms, 8874 A5 forms and 1992 L5 forms; 1223 of these patients were definitely or possibly suffering from syphilis, while the remainder were not suffering from syphilis but showed a deviating serology. Of this remainder, 369 patients had gonorrhoea, and 179 had some other venereal disease. All further processing was confined to patients who had received no antibiotics during the 90 days preceding the first examination. Also excluded from further processing were data on all patients who, anamnestically or according to records available in the department, had pre-

viously suffered from one of the forms of early infectious syphilis. In each chapter it will be specified which part of the total input was studied.

5.8. Follow-up of treated patients

After completion of treatment, blood samples were drawn for serological syphilis tests. This was preferably done also 3, 6 and 12 months after completion of treatment, and subsequently once a year. In actual practice it was not always possible to draw samples on these exact dates; observations on interim gauge-dates are therefore also mentioned. In some cases, several observations were thus made within one gauge-date period. In such cases only the last result was considered.

Two years after treatment for one of the forms of early infectious syphilis, patients were examined again in order to exclude possible symptoms of late symptomatic syphilis. These follow-ups included clinical neurological examination (neurological out-patient clinic, University Hospital Rotterdam; for sailors: department of neurology, Havenziekenhuis, Rotterdam) including CSF studies (cell count, total protein, γ-globulin, Nonne and Pandy tests, gold sol curves and serological syphilis tests), a chest X-ray and an electrocardiogram. The chest X-ray and the electrocardiogram were intended for registration of basic data on the patient involved; possible evidence of cardiovascular syphilis cannot be expected until much later.

In actual practice, only a limited number of patients proved to be still available for follow-up 2 years after completion of treatment. Some of these refused to submit to neurological examination, and this further reduced the data available on this part of the follow-up group. The follow-up period during which a patient adhered to the above described schedule, depended on various imponderables such as change of residence, illness, death, departure to foreign countries (sailors), and on a number of factors that might to some extent be influenced, e.g. obstinacy and lack of understanding on the part of the patient. Although efforts were made to have the socio-medical service for control of venereal diseases approach all syphilis patients who did not come for follow-ups, the study revealed a substantial decrease in the number of patients who did return.

Of the group of 339 patients with early infectious syphilis (both civilians and sailors), only 246 (72%) proved to have returned on gauge-dates 43-90 (i.e. 43-90 days after institution of treatment). On gauge-date

1–1½ year they numbered 143 (42.1%), on gauge-date 2–2½ years 65 (19.1%) and on gauge-date 3–4 years 40 (11.7%).

Other investigators (Perdrup, 1960; Schroeter et al., 1972; Stüttgen and Bartunek, 1973) had similar problems, and likewise lost sight of 50–75% of their syphilis patients in the course of a follow-up period of 1–2 years. The so-called Blue Star Study – a prospective study of the effect of treatment of syphilis patients with penicillin – was started in the USA in 1945 (Bauer, 1951). It attempted to continue a follow-up of the largest possible number of patients over a long period, and succeeded by virtue of considerable efforts (85.1% of patients were still available for follow-up after 8 years) (Shafer et al., 1954). The course of a number of non-treponemal serological tests in this group of patients proved to correlate well with that in a group of patients less intensively followed, and therefore greatly reduced in number in the course of time (Iskrant et al., 1951). In view of this fact, Iskrant et al. (1951) and Shafer et al. (1954) reached the conclusion that patients lost from follow-up for some reason presumably show the same course of serological tests as those who remain available.

In the analysis of our personal observations, we proceeded from the assumption that this also applied to the data we collected.

MEDICAL FACULTY ROTTERDAM
ACADEMIC HOSPITAL ROTTERDAM - DIJKZIGT

DEPT. OF DERMATOLOGY EPIDEMIOLOGY
FILE NUMBER :

NO. A.1.

FILE - NUMBER

0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9

STARTING DATE EXAMINATION = DATE 0

00 10 20 30 day
0 1 2 3 4 5 6 7 8 9
10/ 1 2 3 4 month 5 6 7 8 9
70 71 72 73 74 75 76 77/ 68 69

Patient: male or female
civilian or sailor

Nationality: 100 200 300 400 500
00 10 20 30 40 50 60 70 80 90
0 1 2 3 4 5 6 7 8 9

Age (in years):
00 10 20 30 40 50 60 70 80 90
0 1 2 3 4 5 6 7 8 9

Former Venereal Infections:

Syph. I :
ja 10 20 30 40 year 50 60 70 80 90
no 1 2 3 4 5 6 7 8 9

Syph. II
ja 10 20 30 40 50 60 70 80 90
nee 1 2 3 4 5 6 7 8 9

Other kinds of syph.
ja 10 20 30 40 50 60 70 80 90
nee 1 2 3 4 5 6 7 8 9

Other kinds of syph.
ja 10 20 30 40 50 60 70 80 90
nee 1 2 3 4 5 6 7 8 9

Other venereal diseases:
yes no

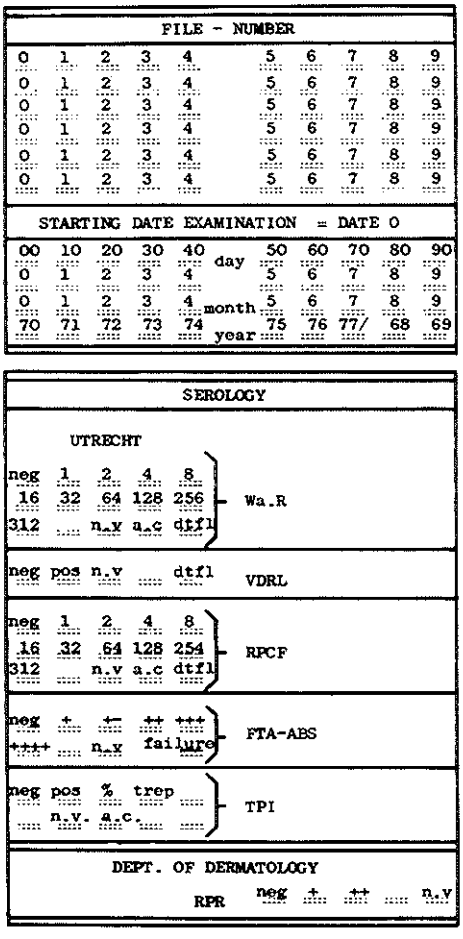
Was an antibiotic/chemotherapeutic therapy prescribed for:
present complaint other illness both no

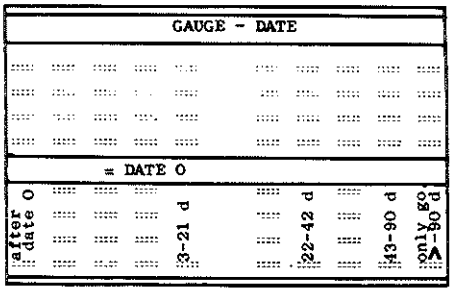
Howmany days before the date of 0:
000 100 200 300 400 500 600 700 800 900
00 10 20 30 40 50 60 70 80 90
0 1 2 3 4 5 6 7 8 9

FIG. 5.1. FORM A I - EPIDEMIOLOGY AND BASIC DATA.

DEPT. OF DERMATOLOGY SEROLOGY

FILE NUMBER NO. A 5





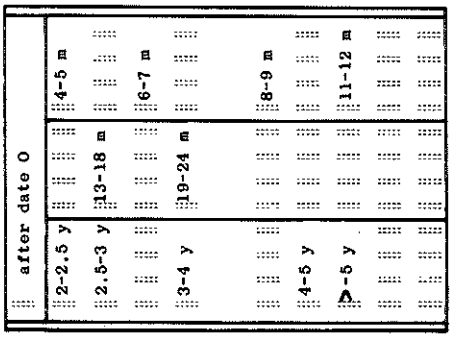


FIG. 5.2. FORM A. 5 - SEROLOGY

FILE NUMBER

DIAGNOSIS/THERAPY

NO. L 5

FILE - NUMBER									
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
STARTING DATE EXAMINATION = DATE 0									
00	10	20	30						
0	1	2	3	4 day	5	6	7	8	9
10/	1	2	3	4 month	5	6	7	8	9
70	71	72	73	74 year	75	76	77//68		69

THERAPY		
Penicillin therapy		
. 30 m. U. pen.	yes	no
. 30 - 15 m.U. pen.	yes	no
. 30 - 15 - 15 m.U.pen	yes	no
. 10.8 m.U. pen.	yes	no
Tetracyclin therapy		
. 60 gr. tetracyclin	yes	no
. 2 x 60 gr. tetracycl.	yes	no
. 3 x 60 gr. tetracycl.	yes	no
Other treatment	yes	no

DIAGNOSIS			
Syph. I seropositive	yes	no	poss
Syphilis II	yes	no	poss
Syphilis recens	yes	no	poss
Syphilis latens tarda	yes	no	poss
Cardiovasc. syphilis	yes	no	poss
Sympt. neuro-syph.	yes	no	poss
Asympt. neuro-syph.	yes	no	poss
End. treponematosis	yes	no	poss
Early cong. syphilis	yes	no	poss
Late cong. syphilis	yes	no	poss
Other stages	yes	no	poss
No syphilis	yes	no	poss
Treated syphilis I	yes	no	poss
Treated syphilis II	yes	no	poss
Treated syph. recens	yes	no	poss
Treated syph. lat. ta.	yes	no	poss
Treated card.vasc.syph.	yes	no	poss
Treated sympt.neuro-s.	yes	no	poss
Treated asympr neuros.	yes	no	poss
Treated end. treponem.	yes	no	poss
Treated early cong.s.	yes	no	poss
Treated late cong.syph.	yes	no	poss
Treated other stages	yes	no	poss
S.no futher classif.	yes	no	poss
Sero - resistence	yes	no	poss

FIG. 5.3. Form L 5 - DIAGNOSIS AND TREATMENT.

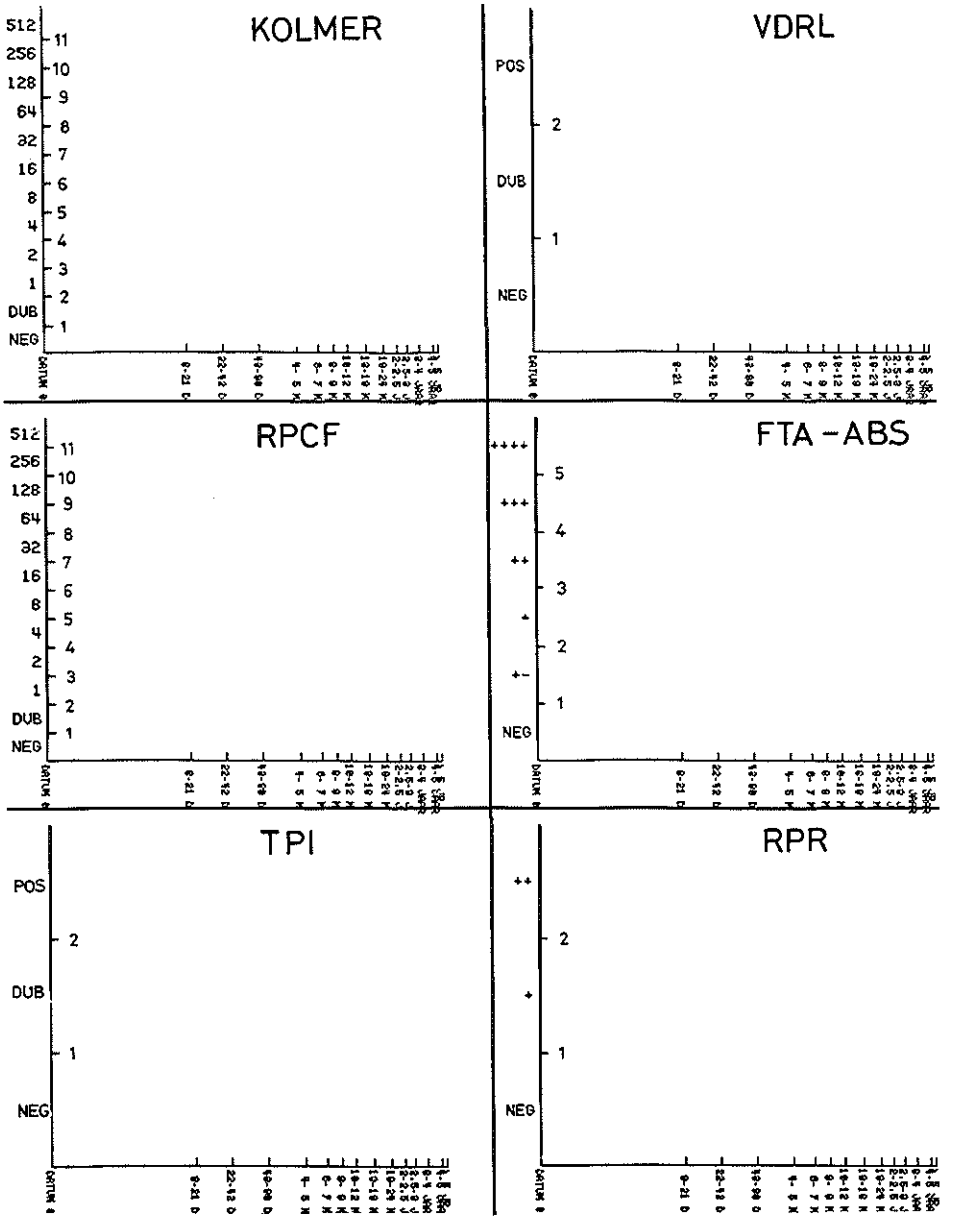


FIG. 5.4 METHOD OF GRAPHICAL REPRESENTATION
X-AXIS : TIME SCALE (LOGARITHMIC)
Y-AXIS : TEST-RESULTS (TITERS GIVEN IN RECIPROKE)
FOR EXPLANATORY REASONS THE GRADUATION OF THE MEDIAN IS SHOWN ON THE INSIDE OF THE X-AXIS

CHAPTER 6

THE COURSE OF VARIOUS NON-TREPONEMAL AND TREPONEMAL SEROLOGICAL TESTS WHEN AN INFECTION WITH T. PALLIDUM IS LEFT UNTREATED

6.1. Introduction

In the course of the development of a bacterial infection both the time of appearance and the degree of persistence of antibodies against the causative agent differ. A large number of factors can be responsible for this:

1) the microorganism involved is a collection of several antigenic determinants, which differ individually in amount and degree of ability to induce an immunological reaction; the antigenic structure of T. pallidum is very complex and comprises at least proteins, lipids, polysaccharides and lipopolysaccharides;
2) the portal of entry, the behaviour of the microorganism and its virulence within the host influence the type and degree of antibody formation; it has been found in test animals that the rate and degree of antibody formation upon intratesticular and intraperitoneal inoculation of T. pallidum depended on the number of treponemata inoculated (Kent et al., 1964).
3) resistance factors characteristic of the individual patient, and possible previous infections with T. pallidum;
4) differences between various antibodies which are specific for a given antigenic determinant, and differences in sensitivity between the various (serological) techniques used to demonstrate the presence of antibodies.

The literature provides only a limited amount of information on the course of serological tests in untreated syphilis. Two large groups of syphilis patients have in the past remained untreated, and have been systematically observed during many years following diagnosis. The follow-up studies on these groups - known in the literature as Boeck's Oslo study and the Tuskegee study - have supplied important information on the late course of untreated human syphilis in terms of clinical and serological aspects of the disease (Bruusgaard, 1929; Gjestland, 1955; Eng and Wereide, 1962; Vonderlehr et al., 1936; Olansky et al., 1956; Rockwell et al., 1964). Olansky (1956) established that in untreated syphilis the

change of non-treponemal tests to negative is largely a function of time. In patients with untreated syphilis of less than 15 years's standing, for example, non-treponemal tests were less frequently found to be negative than in older patients with infections of longer standing. This study, and other investigations (Eng et al., 1962), revealed that the TPI test fails to become negative even after many (sometimes more than 50) years in patients with untreated syphilis or with syphilis treated at a very late stage. Cutler et al. (1952) left patients with primary sero-negative syphilis untreated for a short period, and described the development of reactivity in a number of non-treponemal tests. They pointed out that a certain order of sequence is discernible in this development.

Many investigations have been devoted to evaluation of a newly developed serological test or a particular mode of treatment. In the description of results, data on the patients' serological status before treatment were often reported, although this was not the principal objective of the study in question. These studies invariably concerned only a limited number of serological tests, performed in often poorly defined or undefined groups of patients. In the literature, the results of the various studies are nearly always reported as percentage of positive tests (sero-reactivity) without specifying titre or intensity of the reaction. Only a few authors (Dandoy, 1967; Salo et al., 1967; Fiumara, 1977; 1978) also supplied data on quantitative results of various tests in patients with early infectious syphilis.

For obvious reasons it was impossible to make a longitudinal study of the course of serological tests in untreated syphilis patients. We had to resort to a cross-section method of investigation, using data obtained in retrospect on patients with various stages of syphilis in whom serological testing had been done prior to treatment. It was important that the diagnosis had been established on the basis of well-defined criteria (see Chapter 5) in all these cases, and that several different treponemal and non-treponemal tests had been simultaneously performed.

In this chapter, these data will be used to indicate the course of a number of serological tests to be expected if a venereally acquired infection with T. pallidum were left untreated. The percentage of sero-reactivity is calculated in the same way as the sensitivity of a given test (i.e. the percentage of positive or weakly positive results obtained with a given test in a given group of syphilis patients). The term sero-reactivity is usually used to report the results of an investigation, while the term

sensitivity is more frequently used when the value of a given test is discussed. In this study, too, the serological data obtained before treatment in various disease stages are used to compare the sensitivity of the various tests.

6.2. Material and methods

The serological data obtained on date 0, i.e. before treatment, were used. Table 6.1 shows the distribution of patients by diagnosis, sex, age (mean age ± SD) and civic status (civilian or sailor).
The results per test are presented in graphs as indicated in section 5.6. The duration of the infection is given on the X-axis through the various corresponding clinical stages. Their place (on the arithmetic scale) has been calculated as average interval between the probable moment of infection and the diagnosis. These anamnestic data were available on only a limited number of patients. The intervals are indicated in table 6.2 with the number of patients to whom the data refer. Anamnestic data on the probable moment of infection were not available on the patients with late latent syphilis and with symptomatic neurosyphilis.
The graphs are based on the median of the observations made in the various groups prior to treatment. Since the data on untreated patients were used, these can be accepted as reflecting the course of the results of the various tests without the influence of treatment. As a measure of the range of the individual observations, the first and the third quartile are indicated in the graphs as $x_{0.25}$ and $x_{0.75}$, respectively. The results are also presented as percentage of sero-reactivity in a tabulated form to facilitate comparison with data from the literature.

6.3. Results

6.3.1. Percentages of positive tests in untreated patients with syphilis in various stages

Table 6.3 shows the percentages of positive tests in patients with various stages of untreated syphilis.
Considering solely the percentage of positive tests, the subgroup of sero-changing primary syphilis and the entire group of primary syphilis prove to show the following order of decreasing sero-reactivity:
FTA-ABS > RPR > RPCF > VDRL > Kolmer > TPI.
McNemar's test was applied to determine the significance of these differences in sero-reactivity; the results obtained are presented in table 6.4,

which indicates the following order of decreasing sero-reactivity:
FTA-ABS ≃ RPR > RPCF ≃ VDRL > Kolmer ≃ TPI.
The differences between FTA-ABS and RPR, between RPCF and VDRL and between Kolmer and TPI were not statistically significant.
In secondary syphilis and early latent syphilis, all tests are positive in nearly all patients. Considering solely the percentage of positive tests, there is hardly any difference between the groups of sero-positive primary syphilis, secondary syphilis and early latent syphilis. In the group of late latent syphilis, about 50% of patients show a negative Kolmer and/or VDRL test, 30% have a negative RPR test and almost 20% show a negative RPCF test. The percentage of positive tests in symptomatic neurosyphilis is not distinctly different from that in late latent syphilis.
The significance of differences in sero-reactivity to various serological tests in patients with untreated late latent syphilis was likewise studied. The results are presented in table 6.5, which indicates the following order of decreasing sero-reactivity:
TPI ≃ FTA-ABS > RPCF ≃ RPR > VDRL ≃ Kolmer.
The differences between TPI and FTA-ABS, between RPCF and RPR and between VDRL and Kolmer were not statistically significant. It is to be noted in this context that a positive TPI is one of the criteria of a diagnosis of late latent syphilis; the TPI should therefore be positive in all these patients.

6.3.2. Graphic representation of the course of serological syphilis tests when an infection with T. pallidum is left untreated

The graphs presented in figures 6.1 through 6.6 indicate the course of a number of non-treponemal and treponemal serological tests during an infection with T. pallidum. The data on which these graphs are based, are separately presented per stage and per test in tables 6.6 through 6.11, which also indicate how often a test was not performed and how often the serum proved to be polluted, anti-complementary or treponema-cidal. As already mentioned in chapter 5, not all patients were submitted to all tests, and this explains the differences in total numbers.
In sero-changing primary syphilis and late latent syphilis the range of individual observations ($x_{0.25}$ and $x_{0.75}$) is wide. A closer look at the curves (figs. 6.1 through 6.6) reveals a number of differences, per test and per group of tests, which can be regarded as characteristic.

The non-treponemal tests Kolmer, VDRL and RPR show a similar course with, more specifically, a rapid increase in reactivity from sero-changing primary to sero-positive primary syphilis, followed by a virtually constant positivity up to and including the early latent stage. Next comes a more or less marked decrease in reactivity in late latent syphilis, but there is no further information on the period of time over which the decrease occurs, neither from the literature nor from personal observations. In sero-changing primary syphilis, the median of the Kolmer and the VDRL is within the negative range, whereas that of the RPR is already within the range of 1+ flocculation. In late latent syphilis, the median of the VDRL is negative, that of the Kolmer at titre 1:1, and that of the RPR at 1+ flocculation. The median of the TPI is negative in sero-changing primary syphilis, becomes positive in the sero-positive stage, and remains positive through the later stages. The median of the FTA-ABS is already positive (2+ fluorescence) in sero-changing primary syphilis, and remains positive through all the stages studied.
In late latent syphilis the graphs are drawn horizontal. They are not connected with the indicated position of the median for symptomatic neurosyphilis because only a small part of the patients with late latent syphilis will develop symptomatic neurosyphilis.
In symptomatic neurosyphilis the median of the Komer, VDRL and RPCF tests is significantly higher than that in late latent syphilis, and TPI and FTA-ABS are both positive.

6.4. Discussion

The graphs (figs. 6.1 through 6.6) reveal marked differences in the course of the various serological syphilis tests in untreated infections with T. pallidum. Specifically, there is a marked difference between the course of the non-treponemal tests (Kolmer, VDRL and RPR) and that of the type-specific treponemal tests (FTA-ABS and TPI). The former show a rapid increase in the early stages of the infection, followed by a decrease to weakly positive (Kolmer and RPR) and dubious (VDRL) in the late latent stage. The type-specific treponemal tests, however, remain positive even in the late latent stage. The RPCF, as group-specific treponemal test, would seem to take an intermediate position: there is a decrease in late latent syphilis, but less pronounced than that of the non-treponemal tests.
In the literature we found no comparable graphic representation of the

course of various non-treponemal and treponemal serological syphilis tests in untreated infections with T. pallidum. A publication of the Center of Disease Control, U.S. Public Health Service, USA (VD Program) contains a graph which indicates the reactivity of the VDRL, FTA-ABS and TPI in the course of untreated syphilis (figure 6.7). This graph is based, not on exact data on a group of patients but on impressions concerning the course of the various tests. The use of sero-reactivity in the graph gives no information on individual titres or intensity of reaction. Moore (1949) published a graph indicating the course of non-treponemal tests in untreated human syphilis in titres (figure 6.8). This author, too, however, mentioned that his graph was merely a hypothetical figure, based on "impressions and practical experience".

The studies mentioned in the introduction to this chapter do not distinguish between the results of serological tests in sero-changing syphilis and those in sero-positive syphilis; nor is the mean interval between the moment of infection and diagnosis indicated. Consequently, only data from the literature on results of serological tests in patients with primary syphilis as a total group could be compared with personal observations. In agreement with the findings of other authors (tables 6.12 through 6.14), the non-treponemal tests were positive in the vast majority of patients in all stages of early infectious syphilis except sero-changing primary syphilis. The data from the literature on corresponding tests in the total group of primary syphilis, however, show a fair degree of variation (table 6.12). Apart from the fact that the studies originated from different laboratories, the diagnostic criteria used were not always strictly defined and similar. The Kolmer and RPR tests were performed and compared with treponemal tests in only a small number of these studies. Our own study revealed a higher sensitivity of the RPCF versus the Kolmer and VDRL in primary syphilis (total group), and a high sensitivity of the FTA-ABS and RPR tests; this is in agreement with data from the literature. The sensitivity of the FTA-ABS in sero-changing primary syphilis was found not to differ significantly from that of the RPR. The difference in results of various tests between the two stages of primary syphilis demonstrates that, without this subdivision (as in the various studies found in the literature), a true impression of the course of the reactivity in the earliest stages of syphilis cannot be gained.

In secondary syphilis, all tests were positive in all patients. This is in agreement with the data from the literature (table 6.13). The scanty data

from the literature on serological tests in early latent syphilis are listed in table 6.14. Our own data on sero-reactivity and titres in patients with early latent and late latent syphilis demonstrate – more clearly than is apparent from the literature – the possibility of differentiating between these stages on the basis of results of non-treponemal tests. This differentiation can be of great importance for epidemiology, infectiosity and possible development of late symptomatic syphilis. In late latent syphilis, both the percentage of sero-reactivity and the median titre of the non-treponemal tests show a marked decrease. The treponemal tests (FTA-ABS and TPI) show the highest sero-reactivity in this stage, thus confirming the conclusions of studies of long-untreated syphilis patients mentioned in the introduction. A striking feature is the high degree of sensitivity of the RPR in this stage. Data on this point were not found in the literature. The titres of quantitatively performed tests, specifically Kolmer and RPCF, show a rapid increase during the primary stage; this persists into the secondary stage and is followed by some decrease into the early latent stage, when the median titre of the Kolmer is 1:64, while that of the RPCF is 1:32. These data are in agreement with the available data from the literature (table 6.15). We found no publications reporting on systematic studies of the titres of serological tests in patients with late latent syphilis and various forms of late symptomatic syphilis. We did find statements that the titres of non-treponemal serological tests are generally low in late latent syphilis, whereas they can be very high in some forms of late symptomatic syphilis, e.g. in dementia paralytica and in late symptomatic syphilis associated with gummatous lesions (Stokes et al., 1941; Barniske, 1957; Orlansky and Norins, 1966). A rise in titre of non-treponemal tests in late latent syphilis, therefore, can be indicative of the development of one of the forms of late symptomatic syphilis.

A striking feature is the wide range of test results in late latent syphilis and symptomatic neurosyphilis. This could perhaps be explained by the heterogeneity of these two groups. The diagnosis 'late latent syphilis' is made in part arbitrarily: the infection must have existed more than two years. Theoretically, this implies that this group can include patients whose infection was acquired $2\frac{1}{2}$ years ago as well as patients who acquired it 30 years ago. Apart from this, patients with late latent syphilis showing high titres of non-treponemal tests may include some individuals in the process of developing a not yet diagnosed late symptomatic syphilis. Patients with symptomatic neurosyphilis can differ in activity of the

infection and therefore possibly also in amount of antibodies demonstrable with the aid of non-treponemal tests.
Of the patients with late latent syphilis, 11% had a negative TPI test. This seems to contradict the previously mentioned criterion of a positive TPI for a diagnosis of late latent syphilis. In actual practice, however, one finds that the TPI can give varying results in late latent syphilis, and in some patients can vary between negative, dubious and weakly positive. For a test result reported as negative, the immobilization percentage in such cases is often found to be just below 20% (personal observation). Serological testing is repeatedly done in cases in which late latent syphilis is considered possible. It is consequently possible that the diagnosis is ultimately made partly in view of a positive TPI, even though this test was negative on the date of processing the test results. Obeid-Ruggli (1960) even described this fluctuation of the results of serological tests as characteristic of late latent syphilis.
An illustration of the possible influence of a testing technique is found in the already mentioned higher sensitivity of the RPR versus the VDRL test. Exactly the same (VDRL) antigen (Manual of tests for syphilis 1969) is used in exactly the same amounts in both tests. The principal technical difference between the two tests is that in the RPR carbon particles are added to the antigen solution, which co-agglutinate when flocculation occurs. This co-agglutination is readily visible macroscopically. In the VDRL, the degree of agglutination is microscopically assessed, without any special marker.

6.5. Conclusions

Regarding the course of serological syphilis tests in untreated infections with T. pallidum, a number of microbiological principles apply also to syphilis. This applies in particular to some factors mentioned in the introduction, e.g. diversity of antigens, diversity of antibodies formed, and differences in serological techniques used.
An exact graphic representation of the course of several different serological syphilis tests, using data on well-defined groups of patients and subdivided according to disease stage, gives insight into the value of the various tests and may be helpful in the interpretation of results of serological tests in untreated patients. This type of representation also makes it possible to compare the sensitivity of the various tests in various stages of the disease. In primary syphilis, the VDRL test was significantly more

often positive than the Kolmer, and the following order of decreasing sensitivity was found:

FTA-ABS ≃ RPR > RPCF ≃ VDRL > Kolmer ≃ TPI.

It was found that about 50% of the patients with late latent syphilis had a negative Kolmer and/or VDRL test; 30% had a negative RPR, and nearly 20% had a negative RPCF test. In late latent syphilis, the following order of decreasing sensitivity was found:

TPI ≃ FTA-ABS > RPCF ≃ RPR > VDRL ≃ Kolmer.

In one of the most important forms of late symptomatic syphilis – symptomatic neurosyphilis – it was found that the titres of non-treponemal tests can be substantially higher than those in late latent syphilis. When high titres are found in non-treponemal tests in patients with serological, anamnestic and/or epidemiological indications of syphilis acquired many years earlier, or when titres of non-treponemal tests rise in patients with late latent syphilis, late symptomatic syphilis should be eliminated.

The importance of repeated serological testing of patients showing no clinical symptoms of one of the forms of early syphilis, was demonstrated by the fact that – in a small proportion of the patients with late latent syphilis – repeated TPI tests before treatment were not constantly positive. The above described pattern of a successive turning positive of the various tests in primary syphilis likewise provides an argument in favour of repeated serological testing of patients suspected of syphilis.

Table 6.1
Distribution of patients by diagnosis, sex, age (mean age ±SD) and civic status (civilian or sailor)

	men			women			civilians	sailors
	number	mean age in years	SD	number	mean age in years	SD	number	number
sero-changing primary syphilis	110	30.1	9.0	6	31.4	8.8	57	59
sero-positive primary syphilis	44	31.6	9.8	4	26	6.9	27	21
secondary syphilis	67	31.5	11.0	27	29	9.5	70	24
early latent syphilis	57	32.4	7.5	21	24.7	4.5	53	25
late latent syphilis	81	46.7	15.2	51	48.2	14.3	114	18
symptomatic neurosyphilis	20	56.2	13.7	4	52.2	14.6	23	1

Table 6.2

Interval between moment of infection and diagnosis

stage of disease	number of days (mean)	SD
primary syphilis sero-changing; n = 44	31.9	13.5
primary syphilis sero-positive; n = 17	41.0	14.6
secondary syphilis n = 35	91.0	27.4
early latent syphilis n = 19	225.8	109.7

Table 6.3

Percentage of positive tests in patients with untreated syphilis

	number of patients	Kolmer	VDRL	RPR	RPCF	TPI	FTA-ABS
primary syphilis sero-changing	116	35.0%	48.2%	83.3%	52.2%	23.3%	90.0%
primary syphilis sero-positive	48	100%	100%	97.5%	100%	100%	100%
primary syphilis total group	164	54.3%	63.4%	87.5%	66.4%	46.0%	91.7%
secondary syphilis	94	100%	100%	100%	100%	99.0%	100%
early latent syphilis	78	100%	98.7%	100%	94.8%	96.4%	100%
late latent syphilis	132	52.9%	47.5%	69.2%	81.8%	89.1%	94.1%
symptomatic neurosyphilis	24	68.2%	52.2%		95.5%	94.1%	93.7%

Table 6.4

Comparison of percentages of sero-reactivity in different serological tests in patients with untreated primary syphilis (McNemar's test)
NS = not significant

			P
VDRL	–	Kolmer	< 0.01
RPCF	–	Kolmer	< 0.01
FTA-ABS	–	Kolmer	< 0.001
TPI	–	Kolmer	NS
RPR	–	Kolmer	< 0.001
RPCF	–	VDRL	NS
FTA-ABS	–	VDRL	< 0.001
TPI	–	VDRL	< 0.001
RPR	–	VDRL	< 0.001
FTA-ABS	–	RPCF	< 0.001
TPI	–	RPCF	< 0.001
RPR	–	RPCF	< 0.001
TPI	–	FTA-ABS	< 0.001
RPR	–	FTA-ABS	NS
RPR	–	TPI	< 0.001

Table 6.5

Comparison of percentages of sero-reactivity in different serological tests in patients with untreated late latent syphilis (McNemar's test)

			P
VDRL	–	Kolmer	NS
RPCF	–	Kolmer	< 0.001
FTA-ABS	–	Kolmer	< 0.001
TPI	–	Kolmer	< 0.001
RPR	–	Kolmer	< 0.05
RPCF	–	VDRL	< 0.001
FTA-ABS	–	VDRL	< 0.001
TPI	–	VDRL	< 0.001
RPR	–	VDRL	< 0.10
FTA-ABS	–	RPCF	< 0.02
TPI	–	RPCF	< 0.05
RPR	–	RPCF	NS
TPI	–	FTA-ABS	NS
RPR	–	TPI	< 0.02

Table 6.6

Results of serological tests in patients with sero-changing primary syphilis prior to treatment

Kolmer																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
74	0	6	4	8	6	6	4	4	1	1	1	0	2	114	0.5	0.8	4.0

VDRL																	
neg	dub	pos	nd											tot	$X_{0.25}$	Me	$X_{0.75}$
60	12	44	0											116	0.5	0.9	2.3

RPCF																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
54	1	12	4	19	13	5	3	2	0	0	0	1	2	113	0.5	2.1	4.7

FTA-ABS																	
neg	+–	+	++	+++	4+	nd								tot	$X_{0.25}$	Me	$X_{0.75}$
7	4	19	37	2	1	46								70	2.3	3.1	3.6

TPI																	
neg	dub	pos	trep	nd	ac	tf								tot	$X_{0.25}$	Me	$X_{0.75}$
82	8	17	2	4	1	2								107	0.5	0.6	1.0

RPR																	
neg	+	++		nd										tot	$X_{0.25}$	Me	$X_{0.75}$
16	35	45		20										96	1.2	1.9	2.5

Table 6.7

Results of serological tests in patients with sero-positive primary syphilis prior to treatment

Kolmer																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	1	1	5	6	4	5	10	11	2	3	0	0	48	6.2	8.4	9.6

VDRL																	
neg	dub	pos	nd											tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	48	0											48	2.3	2.5	2.8

RPCF																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	2	4	10	6	5	9	4	8	0	0	0	0	48	4.6	6.4	8.3

FTA-ABS																	
neg	+–	+	++	+++	4+	nd								tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	8	19	0	0	21								27	3.0	3.3	3.7

TPI																	
neg	dub	pos	trep	nd	ac	tf								tot	$X_{0.25}$	Me	$X_{0.75}$
0	8	37	0	3	0	0								45	2.1	2.4	2.5

RPR																	
neg	+	++		nd										tot	$X_{0.25}$	Me	$X_{0.75}$
1	7	32		8										40	2.1	2.4	2.5

Table 6.8

Results of serological tests in patients with secondary syphilis prior to treatment

Kolmer

neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	0	0	0	1	3	3	20	21	25	30	1	1	93	9.3	10.3	11.2

VDRL

neg	dub	pos	nd	tot	$X_{0.25}$	Me	$X_{0.75}$
0	1	93	1	94	2.2	2.5	2.5

RPCF

neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	1	0	7	5	8	20	19	34	0	0	0	1	94	7.1	8.3	9.3

FTA-ABS

neg	+—	+	++	+++	4+	nd	tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	24	29	1	1	40	55	2.6	3.1	3.6

TPI

neg	dub	pos	trep	nd	ac	tf	tot	$X_{0.25}$	Me	$X_{0.75}$
1	1	80	0	10	3	0	82	2.2	2.5	2.5

RPR

neg	+	++	nd	tot	$X_{0.25}$	Me	$X_{0.75}$
0	12	54	29	66	2.1	2.4	2.7

Table 6.9

Results of serological tests in patients with early latent syphilis prior to treatment

Kolmer																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	1	2	4	7	8	11	22	9	9	4	1	0	77	6.7	8.3	9.3

VDRL																	
neg	dub	pos	nd											tot	$X_{0.25}$	Me	$X_{0.75}$
1	2	74	1											77	2.2	2.5	2.5

RPCF																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
4	0	1	2	2	9	14	17	20	8	0	0	1	0	77	6.1	7.4	8.4

FTA-ABS																	
neg	+–	+	++	+++	4+	nd								tot	$X_{0.25}$	Me	$X_{0.75}$
0	0	14	27	4	4	29								79	2.9	3.4	3.8

TPI																	
neg	dub	pos	trep	nd	ac	tf								tot	$X_{0.25}$	Me	$X_{0.75}$
2	0	54	0	21	1	0								56	2.2	2.5	2.5

RPR																	
neg	+	++		nd										tot	$X_{0.25}$	Me	$X_{0.75}$
0	5	30		43										35	2.1	2.4	2.5

Table 6.10

Results of serological tests in patients with late latent syphilis prior to treatment

Kolmer

neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
55	0	9	15	8	12	9	4	2	2	1	0	9	6	117	0.5	2.4	5.1

VDRL

neg	dub	pos	nd	tot	$X_{0.25}$	Me	$X_{0.75}$
64	9	49	10	122	0.5	1.0	2.4

RPCF

neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
21	2	23	25	17	18	3	4	3	0	0	0	8	8	116	2.3	3.5	4.9

FTA-ABS

neg	+–	+	++	+++	4+	nd	tot	$X_{0.25}$	Me	$X_{0.75}$
4	4	20	37	3	0	64	68	2.5	3.2	3.6

TPI

neg	dub	pos	trep	nd	ac	tf	tot	$X_{0.25}$	Me	$X_{0.75}$
9	6	68	1	45	3	0	83	2.1	2.4	2.5

RPR

neg	+	++	nd	tot	$X_{0.25}$	Me	$X_{0.75}$
12	11	16	93	38	0.8	1.7	2.4

Table 6.11

Results of serological tests in patients with symptomatic neurosyphilis prior to treatment

Kolmer																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
7	0	2	1	0	0	0	3	1	3	3	2	1	1	22	0.8	7.3	9.8

VDRL																	
neg	dub	pos	nd											tot	$X_{0.25}$	Me	$X_{0.75}$
11	0	12	1											23	0.5	2.0	2.5

RPCF																	
neg	dub	1	2	4	8	16	32	64	128	256	512	nd	ac	tot	$X_{0.25}$	Me	$X_{0.75}$
1	1	7	1	1	0	1	5	4	1	0	0	1	1	22	2.5	5.0	7.9

FTA-ABS																	
neg	+–	+	++	+++	4+	nd								tot	$X_{0.25}$	Me	$X_{0.75}$
1	1	6	8	0	0	8								16	2.3	3.0	3.5

TPI																	
neg	dub	pos	trep	nd	ac	tf								tot	$X_{0.25}$	Me	$X_{0.75}$
1	1	15	0	6	1	0								17	2.2	2.4	2.7

RPR																	
neg	+	++		nd										tot	$X_{0.25}$	Me	$X_{0.75}$

insufficient data

Table 6.12

Percentage of positive tests (sero-reactivity) in patients with untreated primary syphilis

	number of patients	Kolmer	VDRL	RPR	RPCF	TPI	FTA-ABS
Edmundson et al. (1954)	30		50.0%			33.0%	
Cannefax et al. (1957)	140				61.4%	25.7%	
Moore et al. (1965)	76		50.0%		48.7%	36.8%	80.7%
Deacon et al. (1966)	103		75.7%			53.4%	86.4%
Knox et al. (1966)	37		80.0%			57.0%	87.0%
Förström et al. (1967)	128	76.0%	66.0%		50.0%	24.0%	
Lassus et al. (1967)	62	77.0%	63.0%		28.0%	15.0%	84.0%
Dandoy (1967)	97	74.4%	71.2%				
Salo et al. (1967)	112				41.0%	31.2%	
Tio (1970)	115	47.0%	42.0%	63.0%	67.0%		
Duncan et al. (1974)	77		73.0%				91.0%
Dijckman et al. (1976)	111	63.1%	64.1%				94.6%

Table 6.13

Percentage of positive tests in patients with untreated secondary syphilis

	number of patients	Kolmer	VDRL	RPR	RPCF	TPI	FTA-ABS
Cannefax et al. (1957)	117				84.6%	72.6%	
Sera-studie (1959)	91	100%	100%		98.9%	100%	
Hederstedt et al. (1964)	23	100%				100%	
Falcone et al. (1964)	217		100%	97.2%			
Brown et al. (1964)	148		100%	100%			
Moore et al. (1965)	100		100%	100%			
Knox et al. (1966)	41		100%			100%	100%
Deacon et al. (1966)	121		100%			98.3%	99.2%
Lassus et al. (1976)	14	100%	100%		100%	100%	100%
Förström (1967)	65	100%	100%		89.0%	90.0%	
Salo et al. (1967)	51				72.5%	88.2%	
Dandoy (1967)	84	100%	98.8%				
Tio (1970)	43	93.0%	95.0%	100%	100%		
Dijckman et al. (1976)	56		100%	100%			100%

Table 6.14

Percentage of positive tests in patients with untreated early latent syphilis

	number of patients	Kolmer	VDRL	RPR	RPCF	TPI	FTA-ABS
Magnuson et al. (1949)	106	98.3%				99.1%	
Chester et al. (1953)	172	100%					
Cannefax et al. (1957)	159				86.1%	88.3%	
Dandoy et al. (1967)	67	98.4%	95.5%				
Fiumara (1978)	275			100%			100%

Table 6.15

Results of quantitatively performed serological tests in various stages of syphilis

titre	VDRL (Dandoy, 1967)		Kolmer (Dandoy)		RPCF (Salo e.a. 1967)		RPR (Fiumara 1977)		FTA-ABS (Fiumara 1978)	
	⩽1/8	⩾1/16	⩽1/8	⩾1/16	⩽1/8	⩾1/16	⩽1/8	⩾1/16	2+	3+
primary syphilis	67.0%	33.0%	47.6%	52.3%	81.2%	18.7%	65.3%	34.7%		
secondary syphilis	10.7%	89.2%	4.1%	95.8%	35.2%	64.7%				
early latent syphilis	44.7%	55.2%	25.0%	75.0%			50.9%	49.1%	24.4%	75.6%

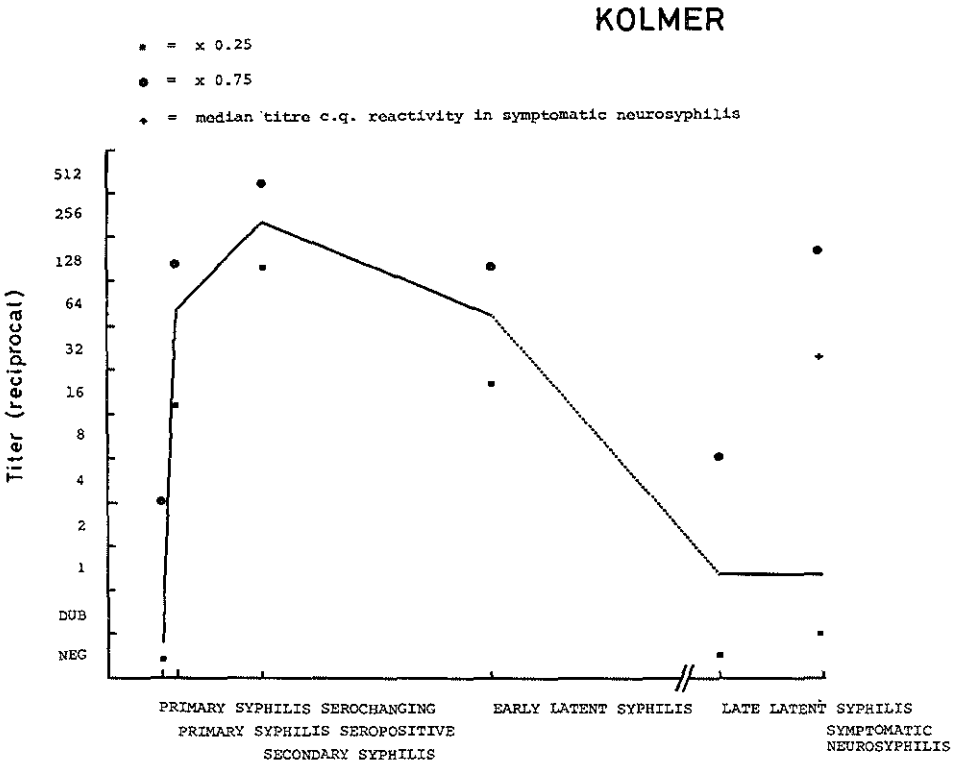


FIG. 6.1. COURSE OF KOLMER IN UNTREATED SYPHILIS

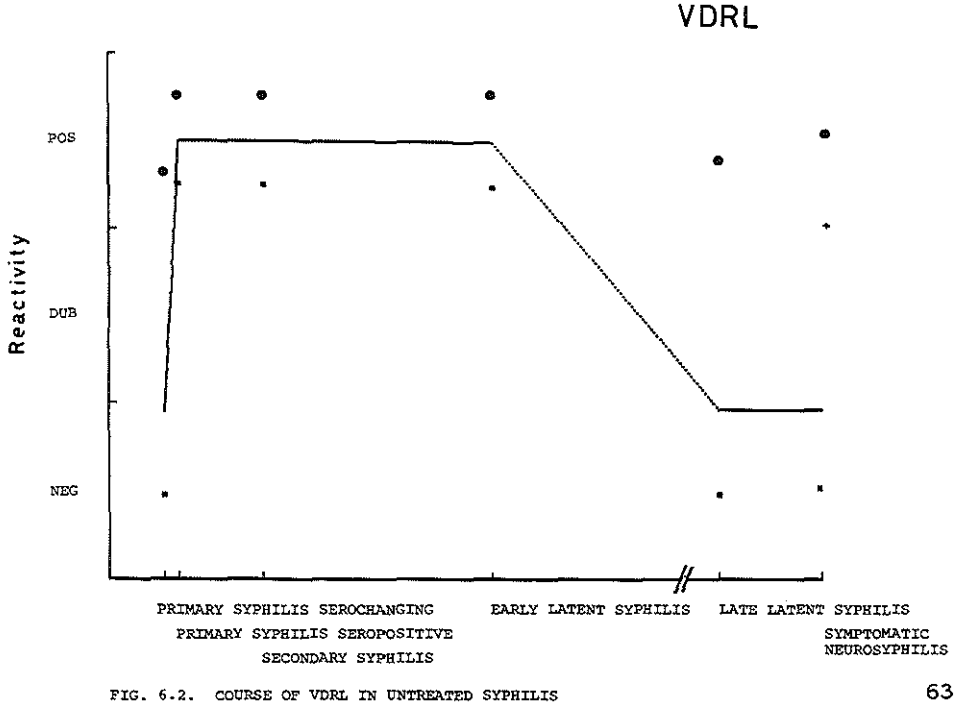


FIG. 6.2. COURSE OF VDRL IN UNTREATED SYPHILIS

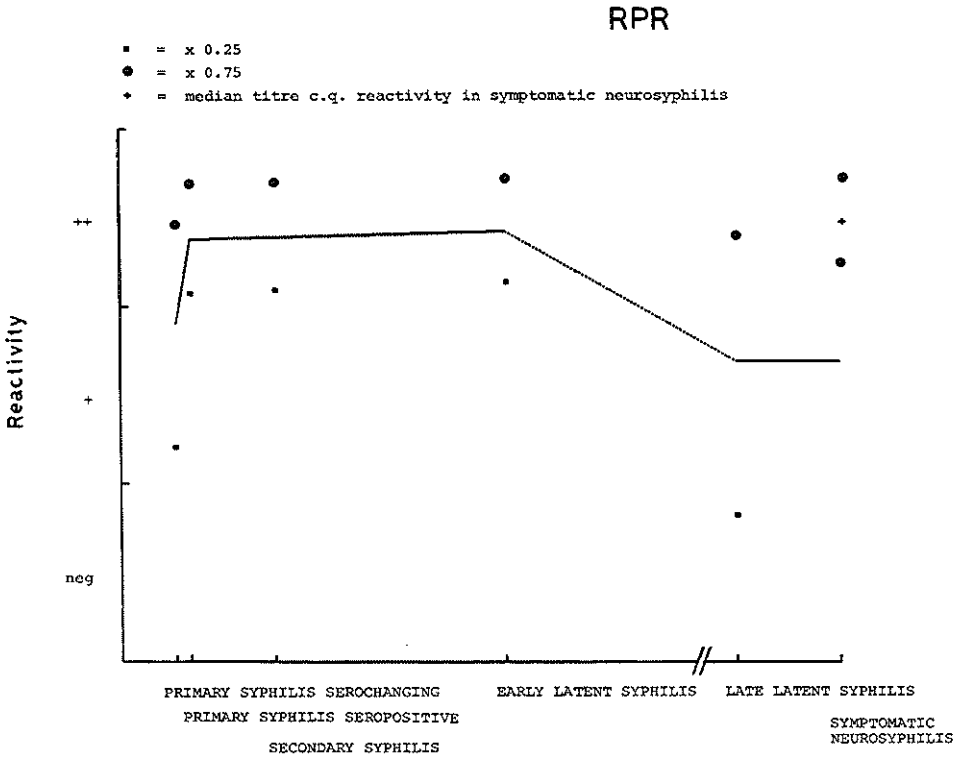


FIG. 6.3 COURSE OF RPR IN UNTREATED SYPHILIS

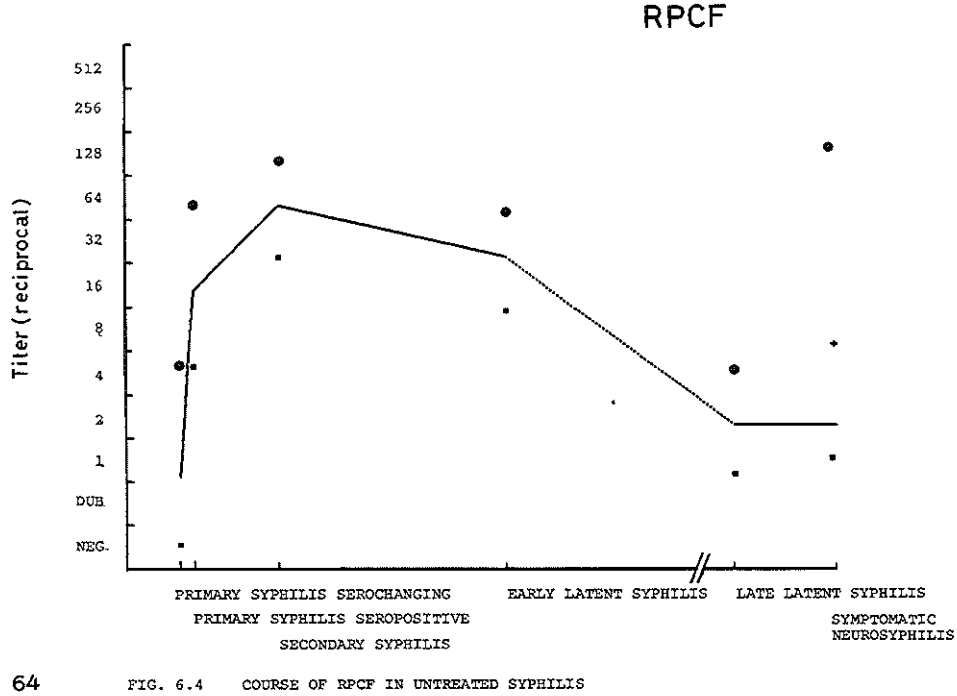


FIG. 6.4 COURSE OF RPCF IN UNTREATED SYPHILIS

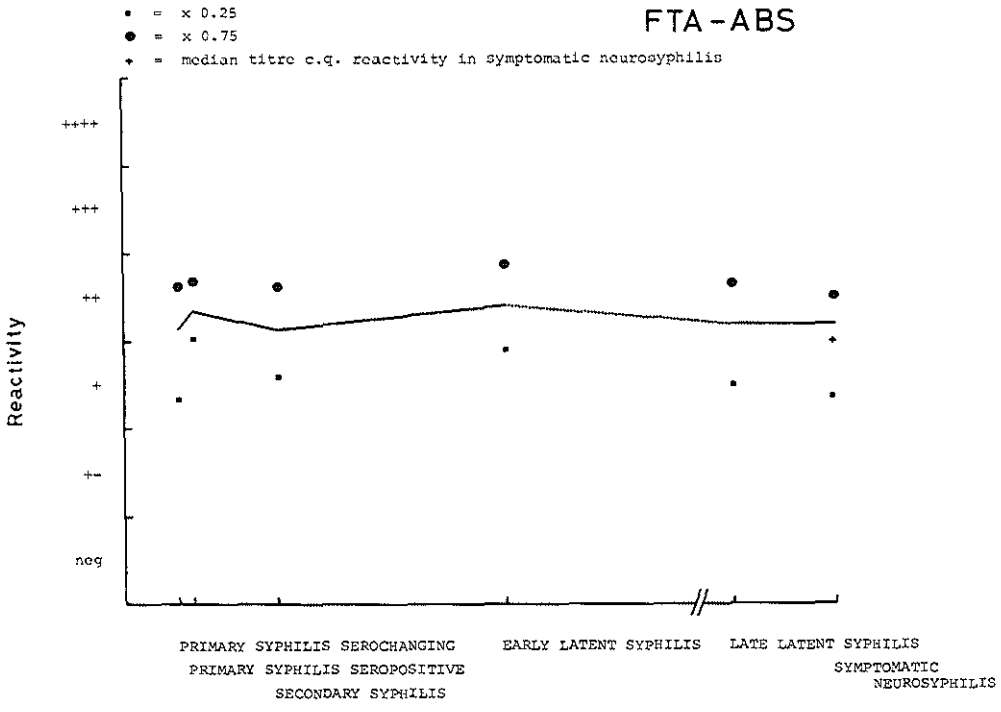


FIG. 6.5. COURSE OF FTA-ABS IN UNTREATED SYPHILIS

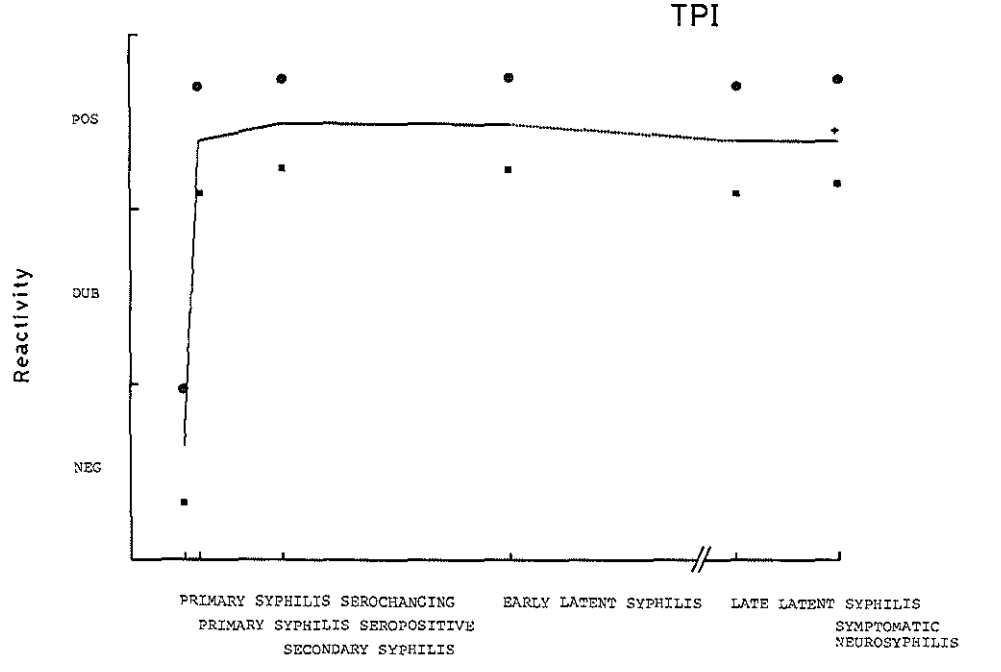


FIG. 6.6 COURSE OF TPI IN UNTREATED SYPHILIS

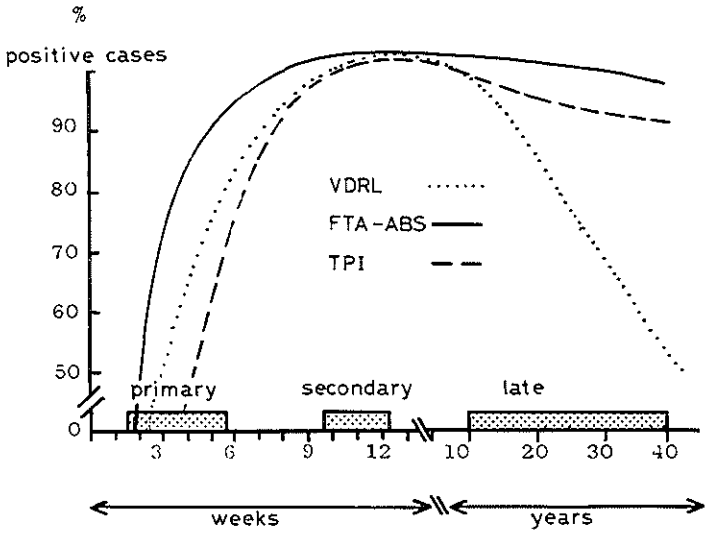


FIG. 6.7 SERO-REACTIVITY IN UNTREATED SYPHILIS
(VD PROGRAM, CENTER FOR DISEASE CONTROL, U.S. PUBLIC HEALTH SERVICE)

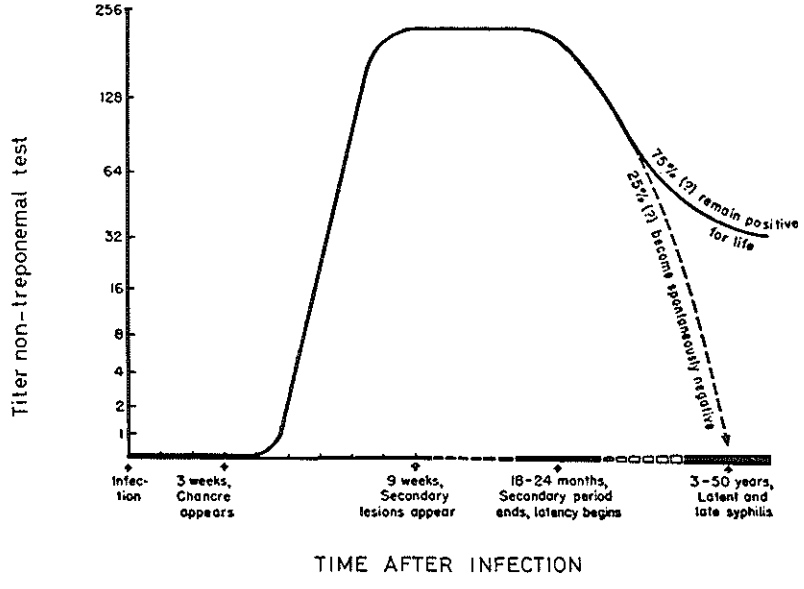


FIG.6.8 COURSE OF TITER NON-TREPONEMAL TEST IN UNTREATED SYPHILIS (MOORE 1949)

CHAPTER 7

INFLUENCE OF PENICILLIN TREATMENT ON THE COURSE OF VARIOUS NON-TREPONEMAL AND TREPONEMAL SEROLOGICAL TESTS IN PATIENTS WITH ACQUIRED SYPHILIS

7.1. Introduction

An important objective in the treatment of syphilis (apart from controlling acute disease symptoms and eliminating infectiousness) is the prevention of late forms of syphilis, and more in particular of the various forms of late symptomatic syphilis.
Criteria for evaluation of the effect of treatment in syphilis patients are limited. In primary and secondary syphilis, the disappearance of the clinical symptoms can serve as criterion. It should be borne in mind, however, that these symptoms also disappear when the patient is left untreated. This spontaneous disappearance of symptoms is followed in 25% of these patients by spontaneous recurrences within the first year. The prevention of these recurrences could be a criterion for the effect of treatment. The prevention of late symptomatic syphilis might also be used as a criterion for the effect of treatment, but studies in this respect are impeded by the long period of observation required.
In follow-ups on treated syphilis patients, serological tests often prove to have the greatest practical importance, both for the doctor and for the patient. In latent syphilis, a possible decrease in reactivity of serological tests is in fact the only direct parameter for the effect of the treatment instituted. In order to avoid superfluous re-treatment of a previously treated patient solely on the basis of serological test results, an insight into the likely course of serological syphilis tests after treatment is required.
The extent to which the results of a serological syphilis test can be influenced by treatment of a syphilis patient, is probably dependent on several factors, e.g. 1) the stage of the disease at the time of treatment (i.e. the duration of the infection and the possible development of immunity), 2) the type of serological test performed, and 3) the type of treatment provided.
Soon after publication of the first description of a serological test which could be effectively used in diagnosing syphilis, it was reported that the

intensity of the reaction varied with the stage of the disease (Landsteiner et al., 1907), and that in many cases the Wassermann complement fixation test quickly became negative in response to treatment. It was also noted that the extent to which the reactivity of a serological syphilis test can be influenced, is dependent on the stage of the disease at the time of treatment. The same was reported for other, subsequently evolved non-treponemal serological syphilis tests (Rein, 1947; Shafer et al., 1954; Huriez and Dujardin, 1959). When the first treponemal test was introduced (TPI test), its reactivity was found to be often less quickly influenced by treatment than that of non-treponemal tests (Crampon and Baelden, 1952; Moore and Mohr, 1952; Jaeger and Delacretaz, 1953; Miller et al., 1954). The same proved to apply to the FTA-ABS test (Deacon et al., 1966; Wood et al., 1967; Atwood et al., 1968; Harner et al., 1968; Hunter et al., 1968; Schroeter et al., 1972; Hunter, 1975).

Different serological tests can show differences in sensitivity, which in part are inherent to the method used and their basic principle. A study of the spontaneous course of the results of several serological tests revealed a distinct difference in the tendency to decrease in reactivity between the non-treponemal tests (Kolmer, VDRL, RPR) and type-specific treponemal tests (TPI, FTA-ABS). It seems plausible that influences which determine this difference between non-treponemal and treponemal tests play a role also in the decrease in reactivity which may occur in response to treatment.

In the course of the first few years after the introduction of penicillin in the treatment of syphilis (Mahoney, 1944), numerous treatment plans were used of which some, in retrospect were certainly not consistent with the basic principles of penicillin treatment for syphilis as mentioned in section 2.10. Therapies not consistent with these principles (Bauer et al., 1947; Barton et al., 1949; Plotke et al., 1951) invariably produced poor results, more specifically a very slow fall of the titres of non-treponemal tests, and a high relapse rate of clinical symptoms. Nevertheless, even after the definition of these principles of penicillin treatment for syphilis there continued to be substantial differences between the treatment plans used in different countries and at different institutes.

Guthe and Idsoe (1968) and Idsoe et al. (1972) published monographs on all results obtained by various therapies as reported by (mainly European) investigators, at least so far as the studies in question fulfilled a number of minimum requirements concerning definition of syphilis stages and

therapy used. Both the serological (tests becoming negative) and the clinical results were assessed in these studies. A striking finding was that few studies mentioned results of treponemal tests after treatment.
The Center for Disease Control, Venereal Disease Control Division, Public Health Service of the American Ministry of Public Health in Atlanta (Thompson et al., 1976) likewise published a comprehensive study of American and English literature on results obtained by various therapies. It was concluded that the efficacy of therapies advised by the WHO for primary syphilis requires further investigation, although no direct evidence was found which indicated the necessity of immediate changes in these recommendations. The question whether there are differences in relative efficacy between the therapies recommended for primary and secondary syphilis, and/or whether an increase of the recommended dosage would enhance efficacy, could not be answered. Due to deficient data from the literature, it could only be recommended that latent syphilis be given more intensive therapy than that advised for primary or secondary syphilis.
This chapter discusses the influence of penicillin treatment on the course of a number of non-treponemal and treponemal serological syphilis tests, on the basis of serological data obtained in retrospect at different intervals after treatment in a number of well-defined groups of patients with various stages of syphilis. The question whether the course of the serological tests differs after two different therapies is also discussed.

7.2. Material and methods

The total groups per diagnosis (sero-changing primary syphilis, seropositive primary syphilis, secondary syphilis, early latent syphilis and late latent syphilis) were subdivided according to treatment given (table 7.1). As already mentioned in Chapter 5, some of the patients were given more than one course of penicillin treatment, dependent on the results of serological tests prior to treatment. In these cases the first course of 31.2 million IU penicillin was followed by a second and sometimes by a third course of 14.4 million IU penicillin. These subgroups comprised only small numbers of patients per treatment and per diagnosis. It was therefore decided for further analysis to regard patients treated with 31.2 million IU, those given 31.2 + 14.4 million IU, and those given 31.2 + 14.4 + 14.4 million IU, as a single group treated with a minimum of 31.2 million IU penicillin over a minimum period of 6 weeks.
On 15th December 1972, i.e. during the period covered by this study,

this high-dosage penicillin therapy (still based on the so-called Bonner salvarsan cures) was abandoned in favour of a course of less large doses of penicillin (10.8 million IU over 6 weeks). This made it possible to compare these two therapies. On the patients (sailors) treated at the Havenziekenhuis, Rotterdam (A. P. Djajadiningrat and Dr E. H. Hermans) with 20.4 million IU penicillin during 14 days, few serological data were available because these patients often departed shortly after release from hospital. Only the pretherapeutic data on these patients were used in analysis.

The study confined itself to the course of serological tests: Kolmer, VDRL, RPCF, FTA-ABS and TPI. Within the period of observation, possible differences in development of late symptoms of syphilis could not be studied. The data on the patients treated with tetracycline, and those on the patients who departed without receiving treatment, were not further analyzed because the numbers involved were too small.

In the discussion of results of serological tests after treatment, the term sero-negativity is used to make comparison with the literature possible. This term refers to the percentage of patients with negative serological findings at a given time.

The patients described above include none with sero-resistance. The data on these patients were separately grouped for computer input under this diagnosis (these patients numbered a total of 14).

7.3. Results

7.3.1. Graphic representation of the course of various serological syphilis tests in response to penicillin treatment, regardless of the dosage and duration of treatment

Figures 7.1 through 7.5 show the course of the serological tests performed during and after penicillin treatment of patients with various stages of syphilis. The tests were performed over a period which ranged from a minimum of 2 to a maximum of 5 years after treatment. Tables 7.2 through 7.6 indicate the number of observations and the value of the median per diagnosis, per test and per gauge-date. For a number of gauge-dates which can be compared with the literature, moreover, the percentage of sero-negative patients was calculated. The results will be discussed per diagnosis and per test.

7.3.1.1. Sero-changing primary syphilis (table 7.2; figs. 7.1 through 7.5):

Kolmer

Before treatment, the median is within the negative range, the test being negative in 65% of patients; 6-7 months after treatment, the test is negative in 100% of patients.

VDRL

Before treatment, the median is within the negative range, the test being negative in 53% of patients; 6-7 months after treatment, the test is negative in all patients. The increase in the median of the VDRL observed 8-21 days after institution of treatment is statistically significant (Wilcoxon's signed-rank test, right-sided).

RPCF

Before treatment, the median is at titre 1:1 and, like that of the VDRL, it increases 8-21 days after institution of treatment. This increase is not statistically significant. The test is negative in all patients investigated 10-12 months after start of treatment.

FTA-ABS

Before treatment, the median is already within the range of 2+ reactivity, the test being still negative in 10% of patients. The median decreases after treatment, and 33% of patients have a negative test after 6-7 months. The median is negative 10-12 months after treatment; 19-24 months after treatment, the test is negative in 73% of patients. All patients still available for follow-up $2\frac{1}{2}$-3 years later show a negative FTA-ABS test.

TPI

Before treatment, the median is within the negative range. Sero-negativity increases from 77% before treatment to 92% 6-7 months after treatment. It is 100% in the patients still available after 10-12 months.

7.3.1.2. Sero-positive primary syphilis (table 7.3; figs. 7.1 through 7.5):

Kolmer

The median before treatment is at titre 1:64 and decreases to negative within 4-5 months. Sero-negativity is 83% after 6-7 months, but does not attain the 100% level until after 19-24 months.

VDRL

The median remains virually unchanged and positive during the first few weeks after treatment. Sero-negativity (0% before treatment) increases to 28%. The median decreases to negative after 4-5 months. Sero-nega-

tivity increases to 87% after 6-7 months, and is 100% of all patients still available after 19-24 months.

RPCF

The median decreases from titre 1:16 before treatment to negative after 8-9 months, when sero-negativity is 60%. Sero-negativity increases to 75% 2-2½ years after treatment.

FTA-ABS

The median lies within the range of 2+ before treatment, and gradually decreases to the range of 1+ after 19-24 months, when sero-negativity is 15%. The median is negative after 2-2½ years, when sero-negativity is 62%. Not in all patients with sero-positive primary syphilis did the FTA-ABS test become negative within the period of observation (2½ years).

TPI

The median varies between positive and dubious during the first 4-5 months after treatment. Sero-negativity after 43-90 days is 50%. The median is negative after 6-7 months, and sero-negativity is 80% after 2-2½ years.

7.3.1.3. Secondary syphilis (table 7.4; figs. 7.1 through 7.5):

Kolmer

The median before treatment is at titre 1:256 and becomes negative after 6-7 months, when sero-negativity is 51%. This subsequently increases to 95% 3-4 years after institution of treatment.

VDRL

The median is negative after 8-9 months; sero-negativity is 93% after 10-12 months, and 89% after 3-4 years

RPCF

The median decreases from titre 1:64 to 1:1 after 13-18 months, when sero-negativity is 30%. Sero-negativity 3-4 years after treatment is 21%.

FTA-ABS

The median fails to decrease over the period of observation (3-4 years), but sero-negativity shows some slight increase, ranging from 5% to 10% after 10-12 months.

TPI

The median decreases to negative 2½-3 years after treatment, while sero-negativity increases from 32% after 10-12 months to 70% after 3-4 years.

7.3.1.4. Early latent syphilis (table 7.5; figs. 7.1 through 7.5):

Kolmer

The median decreases from titre 1:64 to negative after 13–18 months, when sero-negativity is 53%.

VDRL

The median is positive before treatment and decreases to negative after 10–12 months. Sero-negativity is 0% before treatment, 60% after 10–12 months and still 60% after 3–4 years.

RPCF

The median decreases from titre 1:32 before treatment to 1:4 after 6–7 months, but does not become negative within the period of observation (3–4 years). Sero-negativity is 5% before treatment and 10% after 3–4 years.

FTA-ABS

The median is at 2+ before treatment and hardly changes throughout the period of observation (3–4 years after treatment). Sero-negativity remains 0%.

TPI

The median is positive before treatment and hardly changes there-after. Sero-negativity increases to 42% after 19–24 months.

7.3.1.5. Late latent syphilis (table 7.6; figs. 7.1 through 7.5):

Kolmer

The median is at titre 1:1 before treatment, and 4–5 years later attains the borderline between negative and dubious. Sero-negativity is 48% before treatment and virtually unchanged (50%) 4–5 years later.

VDRL

The median is at the borderline between negative and dubious before treatment, and remains there throughout the period of observation. Sero-negativity is 57% before treatment and increases to 67% of the patients still available after 4–5 years.

RPCF

The median is at titre 1:2 before treatment, remains virtually unchanged and is at titre 1:1 after 4–5 years. Sero-negativity is 18% before treatment and likewise remains virtually unchanged throughout the period of observation.

FTA-ABS

The median is within the range of 2+ reactivity before treatment as well

as after. Sero-negativity varies from 5% before treatment to 8% after 3–4 years.

TPI

The median remains within the positive range throughout the period of observation. Sero-negativity remains virtually unchanged.

7.3.2. Comparison of patients with early infectious syphilis treated with different penicillin dosages

So far as the size of the groups after treatment and the number of observations per test permitted, the results of serological tests were compared in patients with sero-changing and sero-positive primary syphilis, secondary syphilis and early latent syphilis, who were treated with different penicillin dosages. The course of the Kolmer, VDRL, RPCF, FTA-ABS and TPI tests after treatment with at least 31.2 million IU penicillin over at least 6 weeks was compared with that after treatment with 10.8 million IU over 6 weeks. The relevant data are presented in tables 7.7 through 7.14.

For the majority of the gauge-dates within a period of observation of up to 2 years after institution of treatment, no significant differences were demonstrable in the course of the serological tests after these two therapies (Wilcoxon's two-sample test, two-sided). In this context it should be pointed out that this was a retrospective study of several groups of patients, without randomization of therapies given. A striking finding was the sometimes very marked increase of the median of some tests shortly after institution of treatment in the patients with sero-changing primary syphilis (VDRL, therapy 31.2 million IU; RPCF, therapy 10.8 million IU). This increase proved to be significant only in the VDRL (Wilcoxon's signed-rank test, right-sided).

The course of the serological tests in patients with late latent syphilis after different therapies was not compared because hardly changes occurred in these tests after treatment (fig. 7.1).

7.4. Discussion

The extent to which the stage of syphilis influences the course of serological tests after treatment is apparent from the graphs and from the sero-negativity percentages after treatment, both for non-treponemal and for treponemal tests. Comparison with observations reported by other authors is possible only on the basis of sero-negativity. Table 7.15

summarizes the results obtained in a number of recent studies of the course of serological tests after treatment, expressed as (percentage of) sero-negativity. We found no publications presenting comparable graphic representations of the course of serological tests in well-defined groups of syphilis patients after penicillin treatment.

For primary syphilis, comparison of sero-negativity percentages as calculated for our patients is possible only with findings reported by Fiumara (1977), who separately studied a group of patients with sero-positive (RPR and FTA-ABS) primary syphilis. The importance of this subdivision in the evaluation of results of serological tests after treatment is demonstrated, not only by the graphic representation of their course but also by the fact that the Kolmer and VDRL tests are negative at 6-7 months after treatment in all patients with sero-changing primary syphilis. In sero-positive primary syphilis, however, this is not the case until after 18-24 months. The other tests. too, show unmistakable differences between these two subgroups of primary syphilis.

Comparison of the results of posttherapeutic serological tests in secondary syphilis with the data from the literature in table 7.15 reveals good agreement in the Kolmer, VDRL, RPCF and FTA-ABS tests. The nontreponemal tests are negative in 50% of patients 6-9 months after treatment, and in 90% after 3-4 years. At this time, 79% of patients still have a positive RPCF and 95% still a positive FTA-ABS test. In secondary syphilis, TPI sero-negativity increases to 70% after 3-4 years. Nielsen and Reyn (1956) collected the results of a large number of studies of the posttherapeutic course of TPI results, regardless of the interval between treatment and TPI testing. They calculated that the TPI was negative after penicillin treatment in 49.5% of patients with secondary syphilis. Only one of the studies referred to by Nielsen and Reyn (Edmondson et al., 1955) specified the interval after treatment. TPI sero-negativity in secondary syphilis was 76% 4 years after treatment, and the authors emphasize that in this group the number of TPI-negative patients distinctly increased between 1 year and 4 years after treatment. Our own findings indicate that it is not until a fairly long time after treatment that TPI tests become negative; calculation of sero-negativity percentages without taking into account the interval after treatment would therefore seem to make little sense.

Remarkably few studies seem to have been devoted to the course of serological tests after treatment of patients with early latent syphilis. On the course of the TPI after treatment of early latent syphilis, the literature stud-

ied provided no data whatever. Personal observations revealed a slight decrease of the median from positive to dubious, with an increase of sero-negativity to 42% after 19-24 months. Hatos (1972) found a negative Kolmer or VDRL test in 58.3% of patients with early latent syphilis 24 months after treatment. This is in good agreement with our findings: 56% Kolmer sero-negativity and 52% VDRL sero-negativity 19-24 months after treatment for early latent syphilis. No satisfactory explanation has been found for the increase of the Kolmer median after $2\frac{1}{2}$-3 years and after 3-4 years, while the median of the other non-treponemal and treponemal tests remained unchanged.

In late latent syphilis, treatment proves to exert virtually no influence on the results of non-treponemal and treponemal serological tests. This was established as early as 1935 for treatment with bismuth and salvarsan (League of Nations 1935), and confirmed also for penicillin treatment (Idsoe et al., 1972).

The course of non-treponemal and treponemal tests after treatment with at least 31.2 million IU penicillin over at least 6 weeks proved not to differ significantly from that after 10.8 million IU penicillin over 6 weeks. Perdrup (1960) concluded from a comparative study that there were no clinical or serological differences after treatment of early syphilis with 6 or with 12 million IU penicillin in daily doses of 600.000 IU procaine-penicillin G in oil with 2% aluminium monostearate (PAM) over 10 or 20 days. After treatment of secondary syphilis with 6 million IU penicillin given as 2.4 or 1.2 million IU benzathine penicillin G (Penidural®) every 3-5 days, Durst et al. (1973) found a more rapid return of the VDRL to sero-negativity than after a single dose of 2.4 or 4.8 million IU of the same agent. Partly in view of the lastmentioned study, the New York City Department of Health and other agencies are now recommending more prolonged penicillin courses with larger doses than those recommended by the Center for Disease Control, Atlanta (USA) and the WHO (Cave, 1975).

The increase of the VDRL median shortly after institution of treatment in patients with sero-changing primary syphilis proved to be statistically significant. Other authors have also reported this (Hoekenga, 1949; Perdrup, 1960; Fiumara, 1964; Wiedmann, 1964; Grabner, 1969), but only Wiedmann (1964) suggested a possible explanation: he thought that these patients must have been treated exactly at the time the serological tests changed, which might explain why this increase in sero-reactivity

is not observed in other stages of syphilis. Another explanation might be that treponemata killed by the treatment release large amounts of antigens within a short time.

Antibody production after an infection with T. pallidum can be influenced by treatment aimed at elimination of the causative agent. As the infection has been of longer duration and therefore the antigenic stimulus has been present longer, antibody production can be more permanently influenced by the development of so-called infection immunity. This infection immunity has been demonstrated experimentally by inoculation of T. pallidum in healthy test subjects as well as in treated and untreated syphilis patients (Magnuson et al., 1956). In persons with a history of infection with T. pallidum, even if treated in the primary stage, immunological behaviour after re-infection proved to differ from that of healthy test subjects. This was apparent in particular from an accelerated humoral response after re-infection, demonstrable both in non-treponemal and in treponemal tests. Those previously treated for one of the forms of early syphilis, nearly always developed clinical signs of a new infection after re-infection, whereas patients with untreated latent syphilis did not.

Investigations done by Fiumara (1977) revealed that, after treatment for re-infection of patients with a history of primary, secondary or early latent syphilis, it took much longer for the non-treponemal tests to show a distinct fall in titre than after treatment of a primary infection.

The question arises whether persistent positivity of serological tests long after treatment in various (mostly later) stages of syphilis might be evidence of 'true' immunity and the development of an immunological memory. In this respect the possibility should be taken into account that the residual titres corresponding with this immunity are maintained by contacts with non-treponemal pathogens, e.g. in the oral cavity.

Some syphilis patients given what is believed to be adequate therapy show no significant decrease in non-treponemal antibodies (present in high titres prior to treatment) during a follow-up period of at least 2 years. This phenomenon is called sero-resistance. Several authors (Moore et al., 1938; Durel et al., 1965; Wigfield, 1965; Ehrmann, 1967) believed that in these patients there might be persistence of the active infection, calling for re-treatment. In the context of this study, the data on the 14 patients with sero-resistance have not been further analysed. In 9 of these 14 patients, all given several courses of penicillin, Schuller (1973) found a high incidence of immunological anomalies consistent with auto-immune

diseases, and liver function disorders. Extensive neurological and internal examinations revealed nothing consistent with late symptomatic syphilis, nor were signs of activity of the original infection found. Analogous to similar anomalies described in the literature in patients with chronic biologically false-positive tests, Schuller suggested that these sero-resistant patients could be suffering from an infection with T. pallidum, superimposed on pre-existent chronic biologically false-positive tests.
Persistent positivity of specific treponemal tests in a number of treated syphilis patients, as observed also in this study, has prompted speculations in the literature about persistence of T. pallidum in the organism after treatment considered to be adequate. Collart (1974) holds that it is not certain that all basic principles of penicillin treatment, e. g. the generation time of T. pallidum, still apply in late stages of syphilis. Several authors (Yoder, 1975; Ritter, 1975; Mohr et al., 1976; Tramont, 1976) described failures with the conventional, internationally recommended doses of penicillin in the treatment of patients with neurosyphilis. In some of these patients the presence of T. pallidum was demonstrable after completion of treatment, and this prompted the advice to treat patients with neurosyphilis by intravenous injection of very large doses of penicillin. The possibility of persistence of T. pallidum, even after treatment, was a reason for adhering to fairly protracted penicillin treatment (as measured by international standards) in all forms of syphilis seen at the dermato-venereological out-patient clinic of the Rotterdam University Hospital.
Luger (1980) presented numerous arguments which cast doubt on the conclusions of studies describing persistence of treponemata after penicillin treatment. He maintained that treponemata might occasionally survive penicillin treatment, but that the clinical consequences of such survivals are of minor importance. Re-treatment should always be effective in such cases.

7.5. Conclusions

A graphic representation of the course of various non-treponemal and treponemal serological tests over a period of 2-5 years after penicillin treatment of patients with various stages of syphilis shows that the stage of the disease at the time of treatment exerts a marked influence on the course of these serological tests. As the disease has existed longer, the decrease in reactivity of the various tests occurs later, or not at all. After treatment of early infectious syphilis, the course of non-treponemal

tests differs markedly from that of treponemal tests. The non-treponemal tests show a marked fall in titre (decrease in reactivity) after treatment in all stages of early infectious syphilis, even though there are differences between the individual stages. The treponemal tests, however, show a much less marked tendency to become negative after treatment. In secondary syphilis, TPI sero-negativity is 67% after $2\frac{1}{2}$-3 years, and the FTA-ABS in fact remains positive in the majority of patients tested 3-4 years after treatment. In early latent syphilis, the number of patients with a negative FTA-ABS does not increase after treatment. The TPI median shows hardly any decrease, but sero-negativity increases to 42% after 19-24 months. In late latent syphilis, penicillin treatment is found not to influence the course of various serological tests over a follow-up period of 4-5 years.

Exact classification of the disease prior to treatment, and taking into account the time of treatment, are of great importance in evaluating results of serological syphilis tests. Otherwise the serological results may be misinterpreted as indicating that treatment has been inadequate. Studies not specifying how long after treatment and in which stage of syphilis the observations were made, can therefore provide no reliable information on the course of a test after treatment.

In non-randomized studies, no significant differences have been found between the course of serological tests after treatment with at least 31.2 million IU penicillin over at least 6 weeks, and that after treatment with 10.8 million IU over 6 weeks.

Table 7.1

Total number of patients, subdivided according to treatment

	sero-changing primary syphilis	sero-positive primary syphilis	secondary syphilis	early latent syphilis	late latent syphilis
31.2 million IU pen.	17	3	8	7	4
31.2 + 14.4 million IU pen.	8	3	5	3	4
31.2 + 14.4 + 14.4 million IU pen.	4	10	18	20	19
total at least 31.2 million IU pen.	29	16	31	30	27
20.4 million IU pen.	30	10	13	7	12
10.8 million IU pen.	42	17	38	27	18
total treated with pen.	102	43	82	64	57
60 g tetracycline *	2	0	7	2	12
different therapy *	4	2	4	7	18
no therapy *	9	3	1	5	45

* The data on these patients were not further analysed in this study.

Table 7.2

Number of patients (n), median (Me) of observations and sero-negativity (% sero-neg.) per test, arranged by gauge-date.
Primary syphilis, sero-changing. Therapy penicillin (total group).

Gauge-date	Kolmer			VDRL			RPCF			FTA-ABS			TPI		
	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg
0	99	0.8	65	101	0.9	53	98	2.1	48	67	3.1	10	92	0.6	77
8 – 21 d	38	0.9		39	1.5		38	3.2		22	3.1		32	0.8	
22 – 42 d	26	0.6		26	0.6		26	0.9		29	2.9		18	0.6	
43 – 90 d	78	0.6	90	81	0.5	91	78	0.7	76	55	2.7	13	41	0.6	78
4 – 5 m	40	0.5		41	0.5		40	0.6		27	2.3		18	0.5	
6 – 7 m	29	0.5	100	30	0.5	100	29	0.6	90	24	1.8	33	12	0.5	92
8 – 9 m	17	0.5		17	0.5		17	0.5		10	2.3		8	0.5	
10 – 12 m	17	0.5		17	0.5		17	0.5	100	15	0.8	60	8	0.5	100
13 – 18 m	25	0.6		25	0.5		25	0.5		22	1.3		15	0.6	
19 – 24 m	19	0.5		18	0.5		18	0.5		18	0.7	73	13	0.5	
2 – 2½ yr	14	0.5		14	0.5		14	0.5		13	0.7		6	0.5	
2½ – 3 yr	5	0.5		5	0.5		5	0.5		5	0.5	100			

Table 7.3

Number of patients (n), median (Me) of observations and sero-negativity (% sero-neg.) per test, arranged by gauge-date. Primary syphilis, sero-positive. Therapy: penicillin (total group).

Gauge-date	Kolmer			VDRL			RPCF			FTA-ABS			TPI		
	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg
0	43	8.4	0	43	2.5	0	43	6.4	0	25	3.3	0	40	2.4	0
8 — 21 d	17	6.6		17	2.5		17	6.2		5	2.8		8	2.2	
22 — 42 d	18	4.0		18	2.4		18	5.5		12	3.3		6	1.0	
43 — 90 d	27	3.9	30	28	2.3	28	27	4.5	18	19	3.4	5	12	1.0	50
4 — 5 m	24	0.7		24	0.8		24	4.0		11	3.4		10	2.0	
6 — 7 m	23	0.6	83	23	0.6	87	23	2.2	43	14	3.4	7	11	0.8	63
8 — 9 m	15	0.6		15	0.6		15	0.8		8	3.3		6	0.7	
10 — 12 m	11	0.8		11	0.6		11	0.9		11	3.2		6	1.0	
13 — 18 m	21	0.6		21	0.6		21	0.8		17	2.6		13	0.8	
19 — 24 m	15	0.5	100	15	0.5	100	15	0.8	60	13	2.3	15	11	0.6	82
2 — 2½ yr	8	0.6		8	0.5		8	0.7	75	8	0.8	62	5	0.6	80

Table 7.4

Number of patients (n), median (Me) of observations and sero-negativity (% sero-neg.) per test, arranged by gauge-date. Secondary syphilis. Therapy: penicillin (total group).

Gauge-date	Kolmer			VDRL			RPCF			FTA-ABS			TPI		
	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg
0	81	10.4	0	82	2.5	0	81	8.4	0	49	3.1	0	71	2.5	0
8 – 21 d	40	9.2		40	2.5		40	7.9		21	3.3		15	2.5	
22 – 42 d	30	7.3		30	2.5		30	6.9		25	3.1		12	2.5	
43 – 90 d	79	6.3	6	79	2.4	11	79	5.9	8	49	3.0	0	29	2.5	3
4 – 5 m	58	3.5		58	2.1		58	4.4		32	3.1		25	2.3	
6 – 7 m	51	1.0	51	51	1.1	49	50	3.8	16	31	3.3	0	22	2.4	
8 – 9 m	29	0.7		29	0.6		29	2.9		22	3.1		17	2.2	
10 – 12 m	28	0.6	89	28	0.5	93	28	2.7		19	2.7	10	19	2.0	32
13 – 18 m	57	0.5		58	0.5		57	2.5	30	45	3.1		42	2.1	
19 – 24 m	32	0.6	92	32	0.6	91	32	3.5		30	3.3	3	28	2.1	32
2 – 2½ yr	19	0.5		19	0.5		19	3.7	26	16	3.1		12	2.0	
2½ – 3 yr	12	0.5		13	0.5		12	3.7	33	13	2.6	8	6	0.7	67
3 – 4 yr	19	0.5	95	19	0.6	89	19	3.7	21	19	3.1	5	10	0.7	70

Table 7.5

Number of patients (n), median (Me) of observations and sero-negativity (% sero-neg.) per test, arranged by gauge-date.
Early latent syphilis. Therapy: penicillin (total group).

Gauge-date	Kolmer			VDRL			RPCF			FTA-ABS			TPI		
	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg
0	64	8.3	0	64	2.5	0	64	7.4	5	46	3.4	0	45	2.5	0
8 – 21 d	27	7.5		28	2.5		27	7.1		19	3.1		9	2.5	0
22 – 42 d	25	5.7		25	2.4		25	6.4		17	3.5		6	2.4	0
43 – 90 d	51	6.1	16	52	2.3	21	52	6.2	0	37	3.1	0	15	2.3	20
4 – 5 m	40	3.7		41	2.2		40	5.0		24	3.3		13	2.5	0
6 – 7 m	43	3.9	32	43	2.0	32	42	4.7	12	31	3.3	0	17	2.1	18
8 – 9 m	21	4.2		22	2.0		22	5.0		13	3.6		10	2.4	10
10 – 12 m	30	2.2	47	30	0.8	60	30	5.0	17	24	3.4	4	14	2.3	21
13 – 18 m	38	0.9	53	38	0.7	71	38	4.3		33	3.3		21	2.3	33
19 – 24 m	25	0.9	56	25	1.0	52	25	4.2	12	25	3.0		12	2.0	42
2 – 2½ yr	19	0.9	53	19	0.9	58	19	4.5		19	2.6		8	2.5	
2½ – 3 yr	6	3.0		6	0.6		6	4.0		6	2.8		–		
3 – 4 yr	9	4.1	33	10	0.8	60	10	3.3	10	10	3.0	0	–		

Table 7.6

Number of patients (n), median (Me) of observations and sero-negativity (% sero-neg.) per test, arranged by gauge-date.
Late latent syphilis. Therapy: penicillin (total group).

Gauge-date	Kolmer			VDRL			RPCF			FTA-ABS			TPI		
	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg	n	Me	sero-% neg
0	50	2.3	48	53	0.9	57	50	3.7	18	37	3.2	5	29	2.4	14
8 – 21 d	12	3.0		17	0.7		12	3.3		11	3.4		11	2.4	
22 – 42 d	27	1.0	52	27	0.9	55	27	3.3	18	22	3.3	9	6	2.4	
43 – 90 d	52	2.6		55	0.9		51	3.3		41	3.1		20	2.5	
4 – 5 m	37	2.5		40	0.8		37	3.6		33	3.2		16	2.4	
6 – 7 m	40	3.5		44	0.9		39	3.0		38	3.1		16	2.4	12
8 – 9 m	21	2.5		22	1.9		21	3.7		16	3.3		12	2.3	
10 – 12 m	25	4.1		25	1.0		25	3.8		25	3.5		12	2.5	8
13 – 18 m	31	2.6		35	0.7		32	3.7		34	3.1		22	2.5	
19 – 24 m	18	1.9		18	0.7		18	4.4		16	3.4		14	2.3	
2 – 2½ yr	12	0.9		12	0.9		12	3.5		12	3.6		6	2.3	
2½ – 3 yr	11	0.9		11	0.9		11	3.7		10	3.0		7	2.4	14
3 – 4	12	0.7		12	0.7		12	3.0		8	3.0	8	10	2.3	15
4 – 5	6	1.0	50	6	0.7	67	6	2.5	17	6	3.7		–		

Table 7.7

Number of patients (n) and median (Me) of observations per test, arranged by gauge-date. Primary syphilis, sero-changing. Therapy: at least 31.2 million IU penicillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	28	0.9	29	1.0	27	3.3	14	2.8	28	0.7
8 – 21 d	17	0.7	18	1.3	17	3.3	8	2.7	14	0.9
22 – 42 d	11	0.5	11	0.5	11	0.8	5	2.8	10	0.6
43 – 90 d	25	0.5	25	0.5	25	0.8	16	2.3	25	0.6
4 – 5 m	21	0.5	22	0.5	21	0.6	8	2.0	11	0.5
6 – 7 m	11	0.5	12	0.5	11	0.6	7	1.2	–	
8 – 9 m	10	0.5	10	0.5	10	0.6	–		–	
10 – 12 m	6	0.6	6	0.5	6	0.5	5	1.5	–	
13 – 18 m	7	0.5	7	0.5	7	0.5	–		–	
19 – 24 m	14	0.5	13	0.5	13	0.5	13	0.7	8	0.5

Table 7.8

Number of patients (n) and median (Me) of observations per test, arranged by gauge-date. Primary syphilis, sero-changing. Therapy: 10.8 million IU penicillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	41	0.7	42	1.0	41	0.8	42	3.2	36	0.7
8 – 21 d	8	1.0	8	1.0	8	4.0	8	3.3	7	0.9
22 – 42 d	13	0.7	13	0.7	13	0.8	13	3.1	7	0.5
43 – 90 d	37	0.6	37	0.6	37	0.6	37	2.9	15	0.7
4 – 5 m	17	0.5	17	0.5	17	0.5	17	2.5	6	0.6
6 – 7 m	16	0.5	16	0.5	16	0.5	16	2.3	10	0.6
8 – 9 m	–		–		–		–		–	
10 – 12 m	8	0.6	8	0.5	8	0.5	8	0.7	5	0.5
13 – 18 m	15	0.7	15	0.5	15	0.5	15	1.5	12	0.6
19 – 24 m	5	0.5	5	0.5	5	0.5	5	0.6	5	0.5

Table 7.9

Number of patients (n) and median (Me) of observations per test, arranged by gauge-date. Primary syphilis, sero-positive. Therapy: at least 31.2 million IU pencillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	16	8.0	16	2.5	16	7.0	–		15	2.4
8 – 21 d	10	6.0	10	2.5	10	6.0	–		–	
22 – 42 d	9	3.7	9	2.1	9	5.9	–		–	
43 – 90 d	16	3.7	16	2.3	16	5.3	7	3.3	7	0.9
4 – 5 m	10	0.7	10	0.7	10	4.1	6	3.3	9	2.1
6 – 7 m	15	0.6	15	0.5	15	2.5	6	3.3	5	0.8
8 – 9 m	9	0.6	9	0.6	9	2.2	–		–	
10 – 12 m	5	0.6	5	0.5	5	4.5	5	3.2	–	
13 – 18 m	10	0.6	10	0.6	10	0.7	6	2.0	–	
19 – 24 m	6	0.5	6	0.5	6	1.0	–		–	

Table 7.10

Number of patients (n) and median (Me) of observations per test, arranged by gauge-date. Primary syphilis, sero-positive. Therapy: 10.8 million IU penicillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	17	8.5	17	2.5	17	6.2	17	3.4	15	2.3
8 – 21 d	–		–		–		–		–	
22 – 42 d	8	1.0	8	2.5	8	4.3	8	3.3	–	
43 – 90 d	10	1.0	11	2.2	10	2.8	11	3.5	5	1.5
4 – 5 m	5	0.8	5	2.2	5	0.8	5	3.5	–	
6 – 7 m	8	0.7	8	0.7	8	1.0	8	3.4	6	0.7
8 – 9 m	5	0.6	5	0.6	5	0.6	4	3.5	–	
10 – 12 m	6	1.0	6	0.6	6	0.7	6	3.3	5	
13 – 18 m	11	0.6	11	0.6	11	0.9	11	3.1	10	0.7
19 – 24 m	9	0.5	9	0.5	9	0.7	9	2.5	7	0.6

Table 7.11

Number of patients (n) and median (Me) of observations per test, arranged by gauge-date.
Secondary syphilis. Therapy: at least 31.2 million IU penicillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	31	10.2	31	2.5	31	8.1	10	2.8	26	2.5
8 – 21 d	20	8.5	20	2.5	20	7.3	7	2.7	–	
22 – 42 d	9	8.1	9	2.5	9	7.4	5	3.0	5	2.5
43 – 90 d	31	6.5	31	2.4	31	6.1	17	2.7	19	2.5
4 – 5 m	20	2.8	20	2.1	20	4.4	12	3.0	19	2.4
6 – 7 m	30	0.9	30	1.2	30	4.0	15	3.1	11	2.4
8 – 9 m	16	0.7	16	0.7	16	3.0	9	3.1	6	2.3
10 – 12 m	17	0.5	17	0.5	17	2.9	8	2.6	13	2.1
13 – 18 m	25	0.6	26	0.5	25	3.1	13	2.7	16	2.0
19 – 24 m	20	0.6	20	0.5	20	3.7	18	3.0	16	2.0

Table 7.12

Number of patients (n) and median (Me) of observations per test, arranged by gauge-date.
Secondary syphilis. Therapy: 10.8 million IU penicillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	38	10.4	38	2.5	38	8.5	37	3.1	35	2.5
8 – 21 d	13	9.7	13	2.5	13	8.2	13	3.5	10	2.4
22 – 42 d	21	7.1	21	2.5	21	6.6	21	3.2	8	2.4
43 – 90 d	30	6.0	30	2.3	30	5.5	30	3.2	8	2.3
4 – 5 m	19	4.8	19	2.1	19	4.4	19	3.2	5	1.5
6 – 7 m	16	1.0	16	0.9	15	3.2	16	3.7	10	2.4
8 – 9 m	13	0.6	13	0.5	13	2.8	13	3.2	11	2.2
10 – 12 m	11	0.6	11	0.6	11	2.3	11	2.7	6	2.0
13 – 18 m	31	0.5	31	0.6	31	1.8	31	3.3	25	2.2
19 – 24 m	12	0.7	12	0.6	12	3.4	12	3.7	12	2.1

Table 7.13

Number of patients (n) median (Me) of observations per test, arranged by gauge-date.
Early latent syphilis. Therapy: 31.2 million IU penicillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	30	8.2	30	2.5	30	7.8	14	3.1	22	2.5
8 – 21 d	14	7.7	14	2.5	14	7.4	7	2.9	–	
22 – 42 d	12	5.8	12	2.5	12	7.0	–		–	
43 – 90 d	22	6.2	22	2.4	22	6.6	17	3.1	8	2.4
4 – 5 m	29	4.1	30	2.3	29	5.4	13	3.3	6	2.5
6 – 7 m	28	4.0	28	2.1	27	5.2	16	3.0	9	2.4
8 – 9 m	16	3.0	17	2.1	17	4.8	8	3.3	7	2.4
10 – 12 m	17	0.9	17	0.8	17	4.9	11	3.3	8	2.3
13 – 18 m	18	0.9	18	0.6	18	4.4	13	2.9	7	2.3
19 – 24 m	20	0.9	20	2.0	20	4.3	20	2.9	9	2.1

Table 7.14

Number of patients (n) and median (Me) of observations per test, arranged by gauge-date.
Early latent syphilis. Therapy: 10.8 million IU penicillin.

	Kolmer		VDRL		RPCF		FTA-ABS		TPI	
Gauge-date	n	Me	n	Me	n	Me	n	Me	n	Me
0	27	8.6	27	2.5	27	7.2	27	3.5	17	2.5
8 – 21 d	8	7.0	8	2.3	8	6.0	8	3.2	5	2.5
22 – 42 d	13	5.5	13	2.3	13	6.2	13	3.6	–	
43 – 90 d	18	6.0	18	2.3	18	5.0	18	3.1	7	2.1
4 – 5 m	10	2.0	10	0.8	10	4.0	10	3.0	6	2.4
6 – 7 m	15	3.7	15	1.5	15	3.5	15	3.5	8	3.1
8 – 9 m	5	5.3	5	1.5	5	5.5	5	4.3	–	
10 – 12 m	12	2.7	12	1.0	12	5.3	12	3.7	6	2.3
13 – 18 m	20	1.0	20	0.8	20	4.2	20	3.5	14	2.2
19 – 24 m	5	0.8	5	0.6	5	3.5	5	3.3	–	

Table 7.15

Results of studies of sero-negativity after treatment of various stages of early syphilis with penicillin (given in millions of International Units = mIU)

	therapy	diag-nosis	test	sero-negativity (%) after months: 2	3	6	12	15	18	24
Förström (1967)	Proc.Pen.G: 7.2 mIU over 12 days	S I	Kolmer		43.0	70.0		96.7		
			VDRL		58.0	90.0		100		
			RPCF		74.0	90.0		93.4		
		S II	Kolmer		13.8	21.5	58.9		77.3	
			VDRL		17.3	21.5	82.4		81.9	
			RPCF		48.3	57.2	53.0		36.4	
Smithurst (1971)	Proc.Pen.G: 10-15 mIU over 10-20 days	S I	Kolmer							96.0
			VDRL							96.0
			RPCF							84.0
	10-40 mIU over 10-40 days	S II	Kolmer							84.6
			VDRL							84.6
			RPCF							76.9
Hatos (1972)	Penidural D/F: 6 mIU over 17 days	S I	Kolmer or VDRL	55.6	69.6	84.2	90.8			
	Penidural D/F: 8.4 mIU over 24 days	S II	Kolmer or VDRL		0	47.7	62.5	76.9	76.3	84.2
		SLR			4.5	22.5	31.4	41.9	50.0	58.3
Capinski (1972)	Proc.Pen.G: 4.2 or 6 mIU	S I	Kolmer		57.0	82.1	92.8			100
	Proc.Pen.G: 6.9 or 12 mIU	S II	Kolmer		20.0	52.0	60.0			84.0
	Penidural: 2.4 or 4.8 mIU over 1-12 days	S I	Kolmer		46.0	76.9	92.3			
	Penidural: 2.4 or 8.4 mIU over 1-3 weeks	S II	Kolmer		31.5	63.1	84.2			100
Durst (1973)	Penidural: 6 mIU over 6-10 days	S II	VDRL				42.5		82.5	100
Luger (1974)	Long-acting Pen: over 14 days	S I	Kolmer		100					
			VDRL		95.4	100				
			FTA-ABS		42.1	69.2	68.7			80.0
		S II	Kolmer		91.0	100				
			VDRL		9.0	27.3	83.3			83.3
			FTA-ABS		0	0	13.3			18.2
Steinmann (1976)	Long-acting Pen: over 2-4 weeks	S I	Kolmer	50.0			90.0		92.0	
		S II	Kolmer			50.0	73.0		72.0	
Fiumara (1977)	Penidural: 4.8 mIU over 2 weeks	S I	RPR	32.6	47.4	79.4	100			
		S II	RPR		34.5	67.0	84.0		96.1	100

S I = primary syphilis; S II = secondary syphilis; SLR = early latent syphilis.

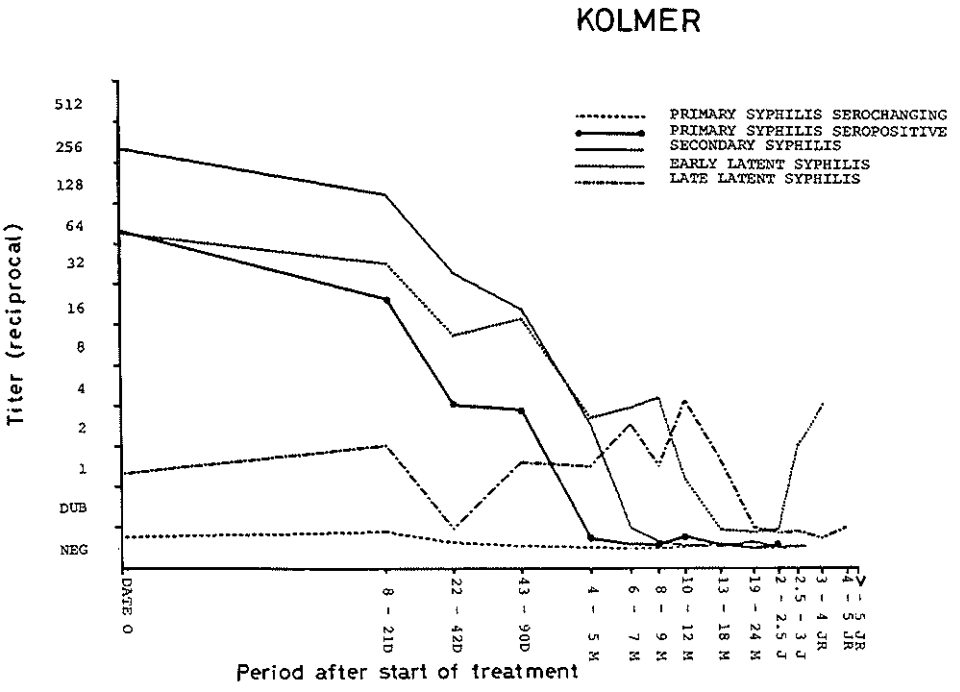


FIG 7.1 COURSE OF KOLMER DURING AND AFTER PENICILLIN-TREATMENT IN DIFFERENT STAGES OF SYPHILIS

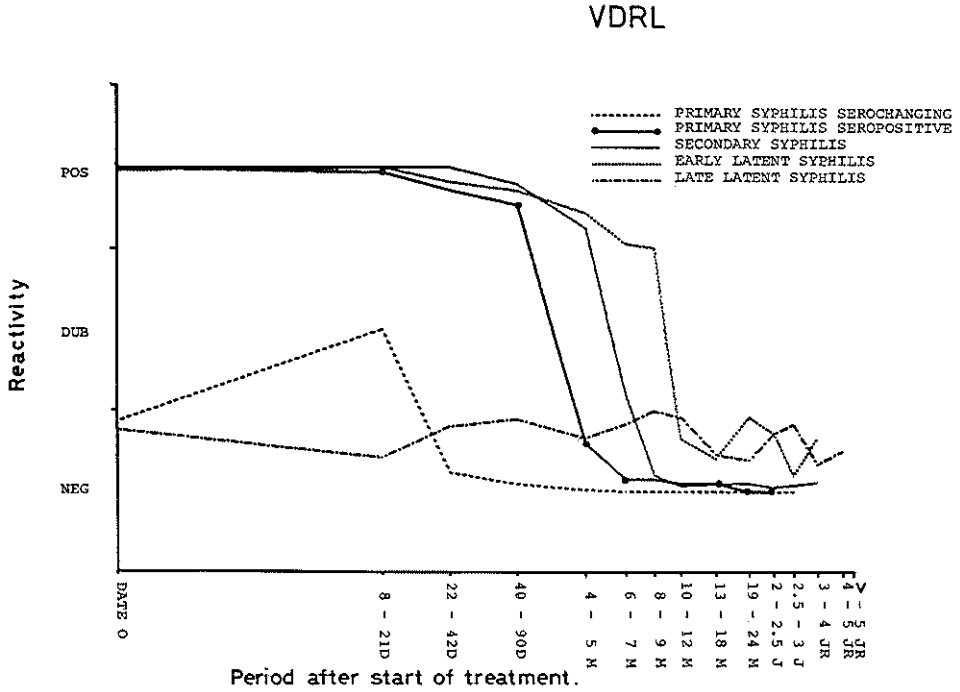
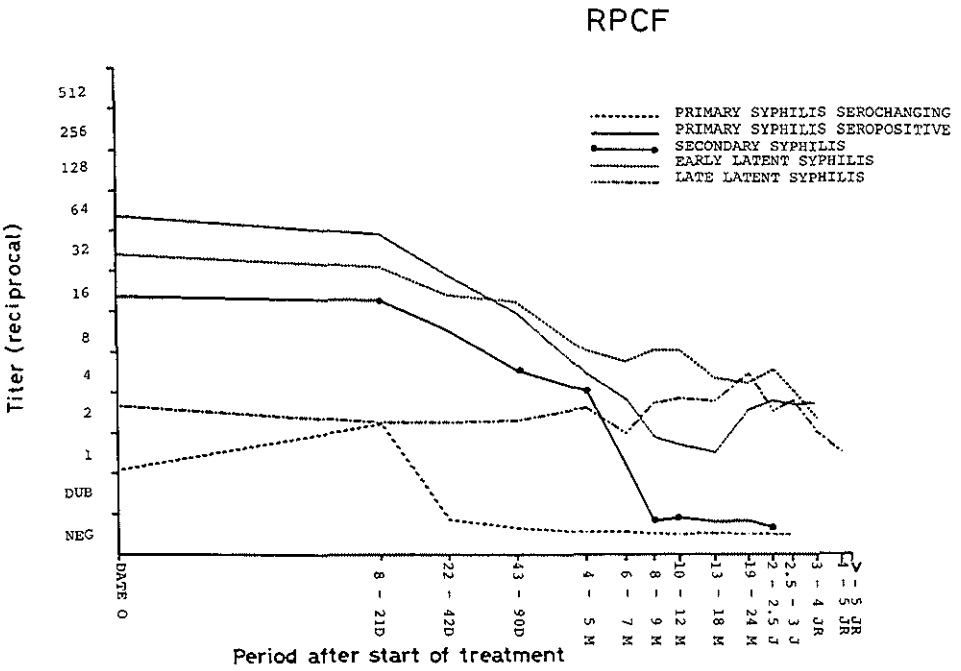


FIG. 7.2 COURSE OF VDRL DURING AND AFTER PENICILLIN-TREATMENT IN DIFFERENT STAGES OF SYPHILIS



FIG, 7.3 COURSE OF RPCF DURING AND AFTER PENICILLIN-TREATMENT IN DIFFERENT STAGES OF SYPHILIS

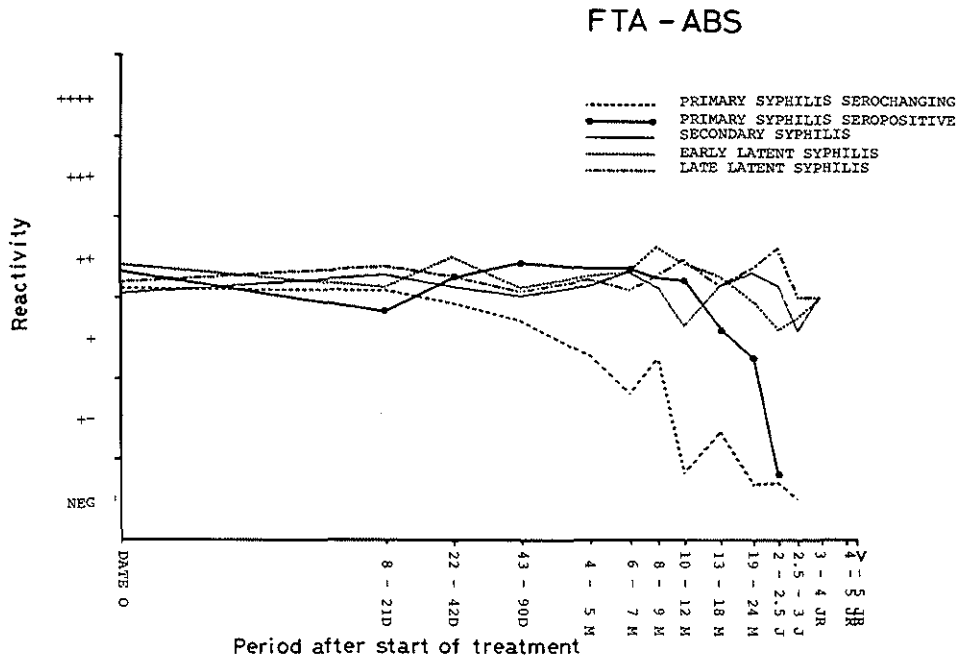


FIG. 7.4 COURSE OF FTA-ABS DURING AND AFTER PENICILLIN-TREATMENT IN DIFFERENT STAGES OF SYPHILIS

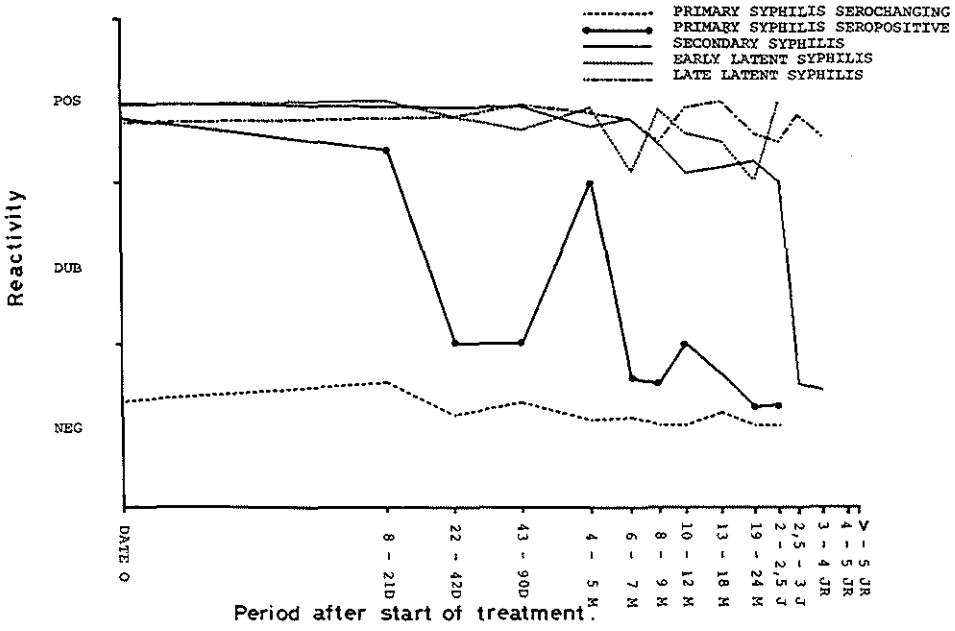


FIG. 7.5 COURSE OF TPI DURING AND AFTER PENICILLING-TREATMENT IN DIFFERENT STAGES OF SYPHILIS

CHAPTER 8

SCREENING TESTS. EFFECTIVENESS OF VARIOUS SEROLOGICAL TESTS OR TEST COMBINATIONS USED IN THE NETHERLANDS FOR DEMONSTRATION OR EXCLUSION OF INFECTION WITH TREPONEMA PALLIDUM IN VARIOUS POPULATIONS

8.1. Introduction

Serological syphilis tests are performed in several different groups:

1) patients suspected of having syphilis on the basis of history, clinical findings or epidemiological data;
2) patients in whom another sexually transmitted disease has been demonstrated, implying the risk of the presence of a simultaneously or not simultaneously acquired infection with T. pallidum as well;
3) persons who have no complaints but present for examination with a view to the possible presence of a sexually transmitted disease;
4) patients suffering from an internal, neurological, psychiatric or other condition, in whom syphilis is to be eliminated or in whom syphilis is suspected as a cause of the symptoms observed;
5) blood donors;
6) pregnant women.

These groups differ, not only in the actual chance that an infection with T. pallidum is present but also in terms of the consequences of missing the diagnosis of syphilis.

An ideal serological syphilis screening test should have a high degree of sensitivity in all stages of the disease. In addition, it should be sufficiently specific, technically simple, quick, capable of being automated, and inexpensive.

A number of properties of various serological tests are schematically summarized in table 8.1. Since none of these tests seems to meet all the requirements, test combinations are often used.

In the Netherlands, serological screening for syphilis is done mostly at district public health laboratories, where several different tests are performed. The guidelines provided by the National Institute of Public Health (Bilthoven) state that at least two tests should be performed at the district laboratories: a flocculation test in combination with either a non-treponemal complement fixation test or a treponemal test. Table 8.2 presents

a survey of serological syphilis tests performed at 19 district public health laboratories (as per 31st August 1978). The VDRL/Kolmer combination was used for screening at 11, and the VDRL/RPCF combination at 2 laboratories. At the request of the referring physician or in view of deviant findings at the first testing, several additional tests might be performed, e. g. the Kolmer (quantitative) and/or RPCF and/or FTA-ABS and/or TPHA tests.
This study attempts to establish whether with the VDRL/Kolmer and VDRL/RPCF combinations all syphilis patients involved would have been identified. Since the TPHA test has recently been recommended also as a screening test (and is meanwhile being used at several laboratories), this test has been included in this study.

8.2. Material and methods

The study encompasses the data on all patients in whom 'syphilis' or 'treated syphilis' had been diagnosed. In each part of the study, only the data on those cases in which all tests to be evaluated had been performed could be considered.
The patients in each diagnostic group were subdivided according to the results of the various serological tests. These were specifically the VDRL/RPCF combination followed by FTA-ABS and TPI, and the VDRL/Kolmer combination followed by FTA-ABS and RPCF. These data were so arranged as to indicate immediately the number of patients with various combinations of test results per diagnosis. In this context it should be borne in mind that the diagnoses were established, not exclusively on the basis of these serological data but on the basis of the criteria defined in Chapter 5 (section 5.1).
Table 8.3 presents data on the sensitivity of the Kolmer, VDRL, RPCF, FTA-ABS, TPI and RPR tests as established in personal observations described in Chapter 6. Since these observations concerned a selected group of patients (i.e. syphilitic patients among those attending an out-patient clinic for venereal diseases), they did not warrant any conclusion concerning the specificity of these tests. The data on the specificity of the Kolmer, VDRL, RPCF and FTA-ABS therefore originate from a study made by Bänffer et al. (1975) among patients attending the dermato-venereological out-patient clinic of the Rotterdam University Hospital. The serological tests were all performed at the bacteriological laboratory of the Rotterdam Municipal Health Service, using the same techniques as

the National Institute of Public Health in Bilthoven. These data are listed in table 8.4, along with data on RPR and TPHA results obtained in a prospective study carried out in collaboration with the National Institute of Public Health in Bilthoven (Menke and Notowicz, 1980). The specificity of the TPI was set at 100%.

The data on the cases of primary and secondary syphilis officially reported in 1979 were supplied by Dr H. Bijkerk (Division of Infectious Diseases, Office of the Chief Medical Officer of Public Health, the Netherlands). Other stages of syphilis are not notifiable in The Netherlands, nor are nation-wide data on these stages otherwise available. According to the American Center for Disease Control (Issue no. 128, Sexually Transmitted Diseases, Statistical Letter, 1980), the ratio between officially reported cases of primary plus secondary syphilis, early latent syphilis, and late latent plus late syphilis per 100,000 USA population was 10: 9.1: 10.6 in 1978. This means that the total number of officially reported cases of syphilis in the USA is three times the number of cases of primary and secondary syphilis. In The Netherlands, the number of reported cases of primary and secondary syphilis was 8.5 per 100,000 population in 1979. Analogous to the situation in the USA, the incidence of all stages of syphilis in The Netherlands was likewise presumed to be three times the number of officially reported cases of primary and secondary syphilis. In 1979 the incidence of primary and secondary syphilis among patients attending the out-patient clinic for venereal diseases of the Rotterdam University Hospital Dijkzigt was 1.8%, versus an incidence of 3.4% for all stages of syphilis taken together.

The data on the incidence of primary and secondary syphilis among blood donors were supplied by F. C. H. A. Kothe, director of the Rotterdam Blood Transfusion Service.

The usefulness of a serological test is determined, not only by its specificity and sensitivity but also by the predictive value of its positive or negative result. This predictive value depends, not only on the specificity and sensitivity of the test but also on the incidence of the disease in the population tested (Vecchio, 1966).

To calculate the predictive value of a positive and of a negative result of a serological syphilis screening test, the following equations were used:

$$PV_{pos} = \frac{\text{number of syphilitics with a positive test}}{\text{total number of persons (syphilitics as well as non-syphilitics) with a positive test}} \times 100$$

$$PV_{neg} = \frac{\text{number of non-syphilitics with a negative test}}{\text{total number of persons with a negative test}} \times 100$$

This means that the predictive value of a positive test result is the percentage of times that a positive test will detect a patient suffering from syphilis, while the predictive value of a negative test result is the percentage of times that a negative test will detect a non-diseased person (Vecchio, 1966).

To calculate the probability that a patient is suffering from syphilis despite a negative test result (i.e. a false-negative test), the following equation was used:

$$P(S^+ / T^-) = \frac{\text{number of syphilitics } (S^+) \text{ with a negative test } (T^-)}{\text{total number of persons with a negative test}} \times 100$$

8.3. Results

Tables 8.5 and 8.6 list the diagnoses corresponding with each combination of serological test results. These data were used for further classification.

8.3.1. Patients with a negative VDRL/RPCF combination

Proceeding from the numbers per diagnosis given in table 8.5, table 8.7 indicates the percentage of the total number of patients in this group and the percentage of the total number of patients with this diagnosis involved in the study. Of the patients with primary syphilis, 18% showed a negative VDRL/RPCF combination. The FTA-ABS was dubious or positive in all these patients. Of the total number of patients with untreated forms of syphilis, 20% showed a negative VDRL/RPCF combination.

8.3.2. Patients with a negative VDRL/Kolmer combination

Proceeding from the numbers per diagnosis given in table 8.6, table 8.8 indicates the percentage of the total number of patients in this group and the percentage of the total number of patients with this diagnosis involved in the study. Of the patients with primary syphilis, 29% showed a negative VDRL/Kolmer combination. Of the total number of patients with untreated forms of syphilis, 37% showed a negative VDRL/Kolmer combination.

The FTA-ABS was dubious or positive in 89% of the patients with primary syphilis, and the RPCF was positive in 30%.

8.3.3. Patients with a negative VDRL/FTA-ABS combination

Proceeding from table 8.5, table 8.9 presents the distribution of patients with a negative VDRL/FTA-ABS combination by diagnosis. It is apparent that the diagnosis would be missed on the basis of a single serological testing in 4% of patients with untreated forms of syphilis.

8.3.4. Predictive values of the Kolmer, VDRL, RPR, RPCF, FTA-ABS, TPI and TPHA tests

The predictive values of positive tests (table 8.10) are widely diverse for different tests and for different disease incidences.
Given a low disease incidence, the predictive values of positive tests are more markedly influenced by minor differences in specificity between different tests (e.g. between Kolmer and VDRL) (table 8.4) than when the disease incidence is higher. The predictive values of a positive Kolmer and VDRL always substantially exceed those of the TPHA and FTA-ABS. Given a specificity of 100% (RPR and TPI), the predictive value of a positive test result is always 100%, regardless of the disease incidence. The predictive value of a negative test result has been calculated only for the group of blood donors, and is about 99.9% for all the tests examined (table 8.11).

8.3.5. Probability of syphilis in the case of a negative test result

Table 8.12 lists for various populations the probability of syphilis in the case of a negative result of various tests. This probability of disease is lowest in the case of a negative TPHA, with FTA-ABS and RPR ranking next. It is highest in the case of a negative TPI, Kolmer and VDRL. These differences occur both at a low and at a relatively high disease incidence.

8.4. Discussion

The choice in favour of the VDRL/Kolmer and VDRL/RPCF combinations discussed here, was made in The Netherlands during the period 1955-1960 on the basis of the tests then available. In The Netherlands, Bekker in particular investigated the value of these tests (Bekker and De Bruyn, 1953; Bekker, 1960). In Great Britain (Sequeira, 1959) and the USA (Carpenter et al., 1960), too, testing schemes were at that time proposed

in which the VDRL, alone or in combination with the RPCF, was considered sufficient for screening purposes.

The groups 1, 2 and 3 mentioned in the introduction, correspond with the out-patient clinic population that provided the patients for this study. It was established that, using the VDRL/RPCF combination in a single testing, syphilis would not have been diagnosed in 20% of the patients with untreated forms of syphilis. With the VDRL/Kolmer combination this would have been the case in 37% of the patients. With the VDRL/FTA-ABS combination the diagnosis would have been missed much less often (in 4% of patients).

In view of these findings it seems justifiable to doubt the effectiveness of the VDRL/Kolmer and VDRL/RPCF combinations for serological testing of the persons in the groups 1, 2 and 3 mentioned in the introduction. Although the diagnosis 'untreated syphilis' would be missed far less often with the VDRL/FTA-ABS combination, this combination cannot be regarded as suitable for screening. This rejection is based on the elaborateness of the fluorescence technique and the already mentioned (subsection 3.3.2.2) occurrence of not readily interpretable fluorescence patterns and sometimes transient weakly positive tests.

The patients of group 4, in whom a form of late symptomatic syphilis is to be excluded, play only a very minor role in this material. Both the VDRL and the Kolmer test were negative in 54% of the patients with asymptomatic and in 12% of those with symptomatic neurosyphilis. This indicates that these tests are insufficient when some form of late symptomatic syphilis is suspected.

In a study of the rheumatoid factor in which the question of the test best suited for its identification was raised, Valkenburg (1974) tried to distinguish between the clinical and the epidemiological criteria to be fulfilled by such a test. An analogous distinction might be made with regard to serological syphilis tests. The clinician regards the results of serological testing as an aid in establishing the diagnosis, and therefore demands a high sensitivity, but also the highest possible rate of negative test results in non-diseased persons. On the other hand, however, the risk of a negative test result in a syphilitic person should be minimal. From the epidemiological point of view, the screening of large population groups expected to include only a small number of patients is primarily intended to trace as many diseased persons as possible, and not to overlook too many. The number of false-positive results of the tests used

should not be too large. These points of view differ only in the degree of importance attached to sensitivity and specificity.
Our personal observations were made in a selected group of patients (in whom syphilis, treated or untreated, had been diagnosed). Our results can therefore not be simply extrapolated to serological studies of the presence of T. pallidum infections in other groups, and particularly in groups in which the expected number of syphilis patients is much smaller.
Applying Bayes rule, an a priori probability, e.g. the incidence of syphilis, can with the aid of laboratory data (specificity and sensitivity of a test) be adapted to the result of that test and turned into an a posteriori probability of disease for a given test result. The thus established predictive value of a test result can then be used in decisions about screening programmes. In addition, the possible occurrence of false-negative tests is also of importance, dependent on the diagnostic situation.
Table 8.10 demonstrates the extent to which the predictive value of a positive test is dependent on the syphilis incidence. In a population with a low syphilis incidence, the predictive values of positive test results are likewise very low. Inter-test differences are marked in that case. The predictive value of a positive test result increases as the syphilis incidence increases. Given a relatively high disease incidence, as among patients attending the out-patient clinic for venereal diseases of the Rotterdam University Hospital, the predictive value of positive non-treponemal tests is likewise very high.
Even with a low disease incidence, the predictive values of positive Kolmer and VDRL tests are the highest, which is to say that the probability of syphilis is highest when these tests are positive.
This might be an important argument in favour of the use of one or several of these tests in epidemiological studies of populations with a low syphilis incidence. But this does not apply to studies of populations with a low syphilis incidence in which a missed diagnosis of syphilis might have serious consequences, as for example in blood donors. Among blood donors, the predictive value of a negative result is about 99.9% for all tests; this is to say that a blood donor with a negative test is almost certainly not suffering from syphilis. Another important point in blood donors is the probability of syphilis in spite of a negative test result.
Table 8.12 shows that this probability is lowest for the TPHA, with FTA-ABS and RPR ranking next. In view of data on Rotterdam blood donors (1978), the Kolmer can be expected to be false-negative in 3.6, and the

TPHA in 0.5 per 100,000 donors. The data on the specificity of the various tests (table 8.4) can be used also to calculate the number of false-positive tests to be expected. This number is 100 per 100,000 persons with the Kolmer, and 1500 per 100,000 with the TPHA test.
For the group of blood donors, the fact that the probability of a false-negative test is seven times as high with the Kolmer as with the TPHA must be weighed against the fact that the probability of a false-positive test is fifteen times as high with the TPHA as with the Kolmer. In view of the possible consequences of a false-negative test result in blood donors, it can be concluded that the TPHA is to be preferred as a screening test for blood donors.
In the serological testing of the other groups mentioned in the introduction, it is up to the clinician to decide whether the sensitivity of a test in various stages of syphilis and the predictive value of a test should not be regarded as very important in these groups as well. If so, then he needs a combination of a test with a high predictive value of a positive result with a test that carries a minimal risk of a false-negative result. As stated earlier, non-treponemal tests have the highest predictive value of positive results. The high sensitivity of the RPR, especially in primary syphilis was shown in Chapter 6. In addition it has a high specificity, which combined with the high sensitivity of the TPHA in all other stages of the disease lead to the conclusion that the RPR/TPHA combination is the combination of choice. Numerous authors have in recent years recommended the use of the TPHA in combination with VDRL or RPR (O'Neill et al., 1973; Young et al., 1974; Luger and Spendlingwimmer, 1974; Kerl et al., 1976; Ehrke et al., 1977; Hagedorn and Naumann, 1979). Verlaeckt et al. (1976) advocated the use of the RPR in combination with both TPHA and FTA-ABS. This advice is based in particular on the possibility which this triple combination affords to trace both false-negative and false-positive results of these two treponemal tests at the very first testing.
The high sensitivity of the RPR in early primary syphilis has already been discussed (section 6.3.1). In many patients with treated syphilis, the TPHA is still positive even after several years (Sequeira and Eldridge, 1973). Although our personal experience in this respect is limited, data from the literature seem to indicate that quantitative performance of the TPHA makes little sense. The graphs indicating the course of serological syphilis tests before and after treatment (Chapter 6 and 7) show that non-treponemal tests, if quantitatively performed, are most suitable as aids

in establishing the correct diagnosis (or in determining the stage of disease). In addition, they can be used in the follow-up after completion of therapy. An important advantage of the use of the RPR/TPHA combination at all district public health laboratories in The Netherlands lies in uniformity, which ensures that referring physicians are no longer confused by a large number of different tests used at different laboratories.

8.5. Conclusions

In view of the results of this study it is evident that the VDRL/Kolmer and VDRL/RPCF combinations widely used in The Netherlands are unsuitable for serological testing of patients suspected of syphilis in view of medical history, clinical symptoms or epidemiological data or in whom syphilis is a possibility or a fear. Moreover, these tests are inadequate also for definite exclusion of syphilis in patients with internal, neurological, psychiatric or other affections.

In determining a choice from various serological syphilis tests for screening purposes, the predictive value of these tests as such is a factor of only limited importance. Only in large-scale epidemiological screening of populations with a low syphilis prevalence, without clinical consequences, will the predictive value mainly play a role in the choice of tests to be used.

In view of the possible consequences of a missed diagnosis of syphilis in blood donors, serological screening of this particular group should be done with a test that carries a minimal risk of a false-negative result. The TPHA meets this requirement, and also other requirements to be made in this context, e.g. possibility of automation and quickness of procedure.

In the other target groups of serological screening mentioned in the introduction, it is in particular a high predictive value of a positive result in combination with high individual sensitivity in various syphilis stages that plays an important role in the choice of the tests to be used. The RPR/TPHA combination meets all reasonable requirements.

An important advantage of the use of the recommended tests at all district public health laboratories would be uniformity, which has long been lacking at this level.

Since the TPHA remains positive for a long time in patients with treated syphilis, particularly in later stages, and often shows no or little inclination to decrease in titre, there should be facilities for performing the non-

treponemal test in question (in this case the RPR) quantitatively at the request of the referring physician. Outside the scope of this discussion of screening tests, mention must be made of the necessity, in the case of a positive test result, of facilities for quantitative performance of the non-treponemal screening test in question for the purpose of further staging of the patient's disease process. For purposes of verification, it should remain possible in The Netherlands to have other treponemal tests (in particular the FTA-ABS and preferably also the TPI) performed.

Table 8.1

Survey of a number of properties of various serological syphilis tests

	Kolmer	VDRL	RPR	RPCF	TPI	FTA-ABS	TPHA
Type of test	Complement fixation	Flocculation	Flocculation	Complement fixation	Immobilization	Immunofluorescence	Haemagglutination
Type of antigen	non-treponemal	non-treponemal	non-treponemal	group-specific treponemal	type-specific treponemal	type-specific treponemal	type-specific treponemal
False-positive tests	possible	possible	possible	possible	very rare	possible	possible
Elaborateness	marked	slight	very slight	marked	very marked	moderate	slight
Duration	2 days	15 min.	10 min.	2 days	2 days	±4 hours	1 - 4 hours
Automation	possible	possible	possible	possible	impossible	partly possible	possible
Sensitivity							
primary syphilis	moderate	high	very high	moderate	low	very high	moderate
other early syphilis	very high	very high	very high	very high	very high	very high	very high
late syphilis	slight	slight	slight	moderate	very high	very high	very high
treated early syphilis	nil	nil	nil	low	moderate	high	very high
treated late syphilis	low	low	low	moderate	very high	very high	very high

Table 8.2

Survey of serological syphilis tests performed at 19 district public health laboratories. (as per 31st August 1978)

Tests minimally performed			Tests performed on indication (deviant findings at first testing or clinical findings)			
VDRL + Kolmer			Kolmer (quantitative)	RPCF	FTA-ABS	TPHA
	11			9	7	1
VDRL + RPCF						
	3		3		2	
VDRL + TPHA						
	2		2	1		
TPHA						
	1		1	1		
VDRL						
	2		2	2		

Table 8.3

Sensitivity of the Kolmer, VDRL, RPR, RPCF, FTA-ABS, TPI and TPHA tests in various stages of syphilis.

	Kolmer[a]	VDRL[a]	RPR[a]	RPCF[a]	FTA-ABS[a]	TPI[a]	TPHA[b]
Primary and secondary syphilis (untreated)	70.9%	76.7%	91.5%	78.8%	95.3%	64.5%	96.3%
All stages of syphilis (untreated)	71.1%	71.6%	89.4%	82.9%	96.2%	75.6%	98.2%

[a] Figures calculated on the basis of data presented in table 6.3.
[b] Data taken from Menke and Notowicz (1980).

Table 8.4

Specificity of the Kolmer, VDRL, RPR, RPCF, FTA-ABS, TPI and TPHA tests.

Kolmer[a]	VDRL[a]	RPR[b]	RPCF[a]	FTA-ABS[a]	TPI[c]	TPHA[b]
99.9%	99.8%	100%	97.6%	96.5%	100%	98.5%

[a] Data taken from Bänffer et al. (1975)
[b] Data taken from Menke and Notowicz (1980)
[c] Presumed specificity

Table 8.5

Combinations of test results (VDRL/RPCF followed by FTA-ABS and TPI)

	VDRL neg.												VDRL pos.											
	RPCF neg.						RPCF pos.						RPCF neg.						RPCF pos.					
	FTA Neg		FTA ±		FTA Pos		FTA Neg		FTA ±		FTA Pos		FTA Neg		FTA ±		FTA Pos		FTA Neg		FTA ±		FTA Pos		
	TPI		TPI		TPI		TPI		TPI		TPI		TPI		TPI		TPI		TPI		TPI		TPI		
Diagnosis	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Tot.
Prim. syph.	—	—	2	—	13	—	2	—	1	—	3	1	1	—	—	—	5	6	—	1	—	—	15	33	83
Sec. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	49	50
Early lat. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	1	29	32
Late lat. syph.	1	—	—	2	—	1	—	—	—	—	—	9	—	—	—	—	—	—	—	—	—	—	—	4	17
Card. vasc. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sympt. neurosyph.	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	3	6
Asympt. neurosyph.	—	—	—	—	—	—	1	—	—	—	2	3	—	—	—	—	—	—	—	—	—	—	—	—	6
Early cong. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Late cong. syph.	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	2
Treated prim. syph.	2	—	3	—	11	—	4	1	—	—	11	2	—	—	—	—	—	—	—	1	—	—	—	1	36
Treated sec. syph.	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	4
Treated early lat. syph.	—	—	—	—	—	1	—	—	1	—	2	3	—	—	—	—	—	—	—	—	—	—	—	2	9
Treated late lat. syph.	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	4	6
Treated card. vasc. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Treated sympt. neurosyph.	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	4
Treated asympt. neurosyph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Treated early cong. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
Treated late cong. syph.	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	3	5
Treated other syph.	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	2
Syph. — unclass.	2	—	4	—	3	10	2	—	—	1	6	26	—	—	—	—	1	2	—	—	—	1	1	34	93
Late lat. syph. or end trep.	2	—	—	—	—	6	—	—	—	1	1	13	—	—	—	—	—	—	—	—	—	1	—	9	33
Total	7	—	9	3	28	19	9	1	2	2	30	63	1	—	—	1	7	9	—	2	—	3	18	175	389

Table 8.6

Combinations of test results (VDRL/Kolmer followed by FTA-ABS and RPCF)

	VDRL neg.												VDRL pos.												
	Kolmer Neg						Kolmer Pos						Kolmer Neg						Kolmer Pos						
	FTA Neg		FTA ±		FTA Pos		FTA Neg		FTA ±		FTA Pos		FTA Neg		FTA ±		FTA Pos		FTA Neg		FTA ±		FTA Pos		
	RPCF		RPCF		RPCF		RPCF		RPCF		RPCF		RPCF		RPCF		RPCF		RPCF		RPCF		RPCF		
Diagnosis	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Tot.
Prim. syph.	1	2	3	1	15	5	—	—	—	—	—	—	1	—	—	—	5	9	—	1	—	—	7	42	92
Sec. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	54	54
Early lat. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	44	47
Late lat. syph.	1	—	1	1	1	9	—	—	1	—	—	5	—	—	—	—	—	1	—	—	—	—	1	11	32
Card. vasc. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sympt. neurosyph.	—	—	—	—	—	1	—	—	—	—	—	2	—	—	—	—	—	—	—		—	—	—	5	8
Asympth. neurosyph.	—	1	—	—	1	5	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	3	13
Early cong. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Late cong. syph.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2
Treated prim. syph.	2	6	3	1	12	14	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	40
Treated sec. syph.	—	—	—	—	—	3	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	4
Treated early lat. syph.	—	—	—	—	1	5	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	2	10
Treated late lat. syph.	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	1	5	9
Treated card. vasc. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Treated sympt. neurosyph.	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	6
Treated asympt. neurosph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Treated early cong. syph.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
Treated late cong. syph.	—	—	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—	3	6
Treated other syph.	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	4
Syph. — unclass.	6	4	4	2	12	32	—	1	—	—	1	17	—	—	—	—	—	3	—	—	—	1	4	48	135
Late lat. syph. or end trep.	2	—	1	1	6	15	—	—	—	—	1	9	—	—	—	—	1	1	—	—	—	1	1	19	58
Total	12	13	13	6	50	92	—	1	1	1	2	42	1	—	—	1	6	15	—	2	1	2	17	243	521

Table 8.7

Distribution by diagnosis of patients with a negative VDRL/RPCF combination (on the basis of data in table 8.5)

diagnosis	number	% of patients with negative VDRL/RPCF (n=66)	% of total number of patients with this diagnosis	number of patients with FTA-ABS ± or pos.
primary syphilis	15	23%	18% (n=83)	15
late latent syphilis	4	6%	22% (n=18)	3
late latent syphilis or latent endemic treponematosis	8	12%	25% (n=32)	6
unclassifiable syphilis	19	29%	20% (n=93)	17
untreated syphilis total	46	70%	20% (n=226)	41
treated prim. syphilis	16	24%	29%	14
treated other forms of syphilis	4	6%	13% (n=31)	4

Table 8.8

Distribution by diagnosis of patients with a negative VDRL/Kolmer combination (on the basis of data in table 8.6)

diagnosis	number	% of patients with negative VDRL/Kolmer (n=186)	% of total number of patients with this diagnosis	number of patients with FTA-ABS ± or pos.	number of patients with RPCF pos.
primary syphilis	27	15%	29% (n=92)	24	8
late latent syphilis	13	7%	41% (n=32)	12	10
late latent syphilis or latent endemic treponematosis	25	13%	43% (n=59)	23	16
asympt. neurosyph.	7	4%	54% (n=13)	6	6
sympt. neurosyph.	1	—	12% (n=8)	1	1
late cong. syph.	1	—	50% (n=2)	1	1
unclass. syph.	48	26%	39% (n=122)	38	38
untreated syph. total	122	66%	37% (n=328)	105	111
treated prim. syph.	38	20%	97% (n=39)	30	21
treated other forms of syphilis	15	8%	36% (n=41)	14	10

Table 8.9

Distribution by diagnosis of patients with a negative VDRL/FTA-ABS combination (on the basis of data in table 8.5)

diagnosis	number	% of total number of patients with this diagnosis
primary syphilis	2	2% (n=83)
late latent syphilis	1	5.5% (n=18)
late latent syphilis or latent endemic treponematosis	2	6% (n=32)
unclassifiable syphilis	4	4% (n=93)
asymptomatic neurosyphilis	1	17% (n=6)
untreated syphilis, total	10	4% (n=232)
treated primary syphilis	7	13% (n=55)

Table 8.10

Incidence of syphilis and predictive values of positive Kolmer, VDRL, RPCF, FTA-ABS and TPHA results [a]

Population	Incidence	Predictive values (in %)				
	(in %)	Kolmer	VDRL	RPCF	FTA-ABS	TPHA
Netherlands, 1979:						
primary and secondary syphilis[b]	0.0085	5.70	2.95	0.30	0.23	0.55
syphilis, all stages[c]	0.0255	15.31	8.91	0.83	0.70	1.64
Amsterdam, 1979:						
primary and secondary syphilis[b]	0.098	41.02	27.33	3.12	2.60	6.03
Rotterdam, 1979:						
primary and secondary syphilis[b]	0.0303	17.69	10.41	0.98	0.82	1.95
Utrecht, 1979:						
primary and secondary syphilis[b]	0.019	11.87	6.79	0.62	0.51	1.22
The Hague, 1979:						
primary and secondary syphilis[b]	0.0098	6.50	3.62	0.32	0.26	0.63
Rotterdam University Hospital Out-patient Clinic for Venereal Diseases, 1979:						
primary and secondary syphilis[d]	1.8	92.85	87.54	37.56	33.29	54.55
syphilis, all stages[d]	3.4	96.15	93.10	53.61	48.94	69.74
Rotterdam blood donors, 1978[e]	0.0125	8.14	4.57	0.41	0.34	0.81

a) Given a RPR and TPI specificity of 100%, the predictive value of positive RPR and TPI results is always 100%, regardless of the disease incidence.

b) Data based on the number of officially reported cases of primary and secondary syphilis under the Infectious Diseases Control and Disease Cause Detection Act (1976).

c) Incidence estimated on the basis of the ratio between primary/secondary syphilis and other stages of syphilis in the USA (1979).

d) Data from out-patient clinic attendance records.

e) Data supplied by F.C.H.A. Kothe, director of the Rotterdam Blood Transfusion Service.

Table 8.11

Predictive values of negative Kolmer, VDRL, RPR, RPCF, FTA-ABS, TPI and TPHA results in blood donors of the Rotterdam Blood Transfusion Service (1978).

	Predictive values (in %)						
Prevalence of syphilis (in %)	Kolmer	VDRL	RPR	RPCF	FTA-ABS	TPI	TPHA
0.0125	99.9964	99.9971	99.9993	99.998	99.9996	99.997	99.9997

Table 8.12

Probability ($P(S^+/R^-)$) that a patient is suffering from syphilis despite a negative test result in various populations (in %)

Population	Incidence prim./sec. syphilis (in %)	Kolmer	VDRL	RPR	RPCF	FTA-ABS	TPI	TPHA
Netherlands, 1979	0.0085	0.0025	0.002	0.0007	0.002	0.0004	0.003	0.0003
Dept. of derm. venerology, Rotterdam University Hospital, 1979	1.80	0.53	0.43	0.15	0.40	0.09	0.64	0.07
Rotterdam blood donors, 1978	0.0125	0.0036	0.0029	0.001	0.0027	0.0006	0.0044	0.0005

SUMMARY

This thesis discusses the results of a study of various aspects of a number of serological syphilis tests.

Chapter 1 outlines the facts that prompted this study. Specifically, these were the problems encountered in actual practice in the interpretation of results of serological syphilis tests. These problems are due in part to inadequate knowledge, and partly also to the fact that a fair number of different tests are in use in The Netherlands.

Chapter 2 presents a survey of clinical and therapeutic aspects of syphilis.

Chapter 3 presents a survey of serological syphilis tests on the basis of data from the literature, proceeding from the division of these tests into non-treponemal and treponemal tests. This division is based on the origin of the antigen used in these tests. The various tests performed in The Netherlands receive special attention. In addition, the concepts of anti-complementarity and biologically false-positive tests are discussed.

Chapter 4 formulates the specific questions on which this study focuses:

1) Which course do various non-treponemal and treponemal serological tests take when a venereally acquired infection with T. pallidum is left untreated, and when it is treated with penicillin, and are there differences after treatment according to two different treatment plans?
2) Can two test combinations widely used in The Netherlands be regarded as effective in the detection of syphilis, and to which extent might the TPHA test contribute to this?

Chapter 5 discusses the diagnostic criteria applied, serological techniques, treatment, computer processing, statistical methods, mode of graphic representation, and the follow-up on treated patients.

Chapter 6 describes the course of a number of non-treponemal and treponemal serological tests during an untreated infection with T. pallidum. The graphs indicating this are based on the median of the observations in the various groups before treatment. The percentage of positive tests (sero-reactivity) of the various tests in different disease stages is also calculated. In primary syphilis the VDRL sero-reactivity significantly exceeds that of the Kolmer test, and the following order of decreasing sensitivity is noted:

FTA-ABS $\simeq$ RPR $>$ RPCF $\simeq$ VDRL $>$ Kolmer $\simeq$ TPI.

The FTA-ABS and RPR are the most sensitive tests; the difference between these two tests is not significant.
The order of decreasing sensitivity in late latent syphilis is:
TPI ≃ FTA-ABS > RPCF ≃ RPR > VDRL ≃ Kolmer.
The graphs reveal marked differences in course between the various serological syphilis tests. In particular there is a distinct difference in course between the non-treponemal tests (Kolmer, VDRL and RPR) and the type-specific treponemal tests (FTA-ABS and TPI).
Mention is made of the occurrence of high titres in non-treponemal tests in patients with symptomatic neurosyphilis. Arguments are marshalled to demonstrate the importance of repeated serological testing when syphilis is suspected.

Chapter 7 presents graphs showing the course of the Kolmer, VDRL, RPCF, FTA-ABS and TPI tests over a period of 2-5 years after treatment with penicillin. Mention is made of marked differences again found between non-treponemal and treponemal tests in patients with early infectious syphilis. In late latent syphilis, penicillin treatment proves not to influence the course of the tests over a period of observation of 4-5 years. As the disease has been of longer duration, the reactivity of the various tests diminishes later, or even not at all. An exact classification of the disease prior to treatment, and taking into account the time of treatment, are of great importance in the evaluation of results of serological syphilis tests. In a non-randomized study, no significant differences were found between the course of serological syphilis tests after treatment with at least 31.2 million IU penicillin over at least 6 weeks, and that after treatment with 10.8 million IU penicillin over a 6-week period.

Chapter 8 discusses the question whether a few test combinations widely used in The Netherlands - specifically the VDRL/RPCF and the VDRL/Kolmer combination - are effective in demonstrating or excluding syphilis. In this context some important properties of the various serological tests are presented in tabulated form. The TPHA test, now in use also at several laboratories, is included in this study. It is demonstrated that, with the two test combinations mentioned, the diagnosis of syphilis would have been missed in 20.3% and 37.2%, respectively, of the syphilis patients considered in this study. Efforts are made to facilitate the choice of serological tests for screening purposes by calculating the predictive values of positive test results. Particularly in dealing with blood donors, it is

important also to know the risk that a donor is suffering from syphilis despite a negative test result. It is concluded that this risk is smallest with the TPHA, and that consequently this test can be recommended for selection of blood donors. For serological syphilis testing of other groups, the use of the TPHA in combination with a non-treponemal test, preferably the RPR, is recommended. Emphasis is placed on the important advantage of uniformity of the tests performed at various district public health laboratories; this can eliminate a possible source of confusion and misunderstanding.

SAMENVATTING

In dit proefschrift worden de resultaten besproken van een onderzoek naar verschillende aspecten van een aantal serologische syfilisreacties.

In hoofdstuk 1 is aangegeven wat tot dit onderzoek aanleiding heeft gegeven. Met name zijn dit de in de praktijk gebleken problemen bij de interpretatie van resultaten van serologische syfilisreacties.
Deze zijn deels te wijten aan gebrek aan kennis en anderdeels ook aan het vrij grote aantal verschillende reacties dat in Nederland verricht wordt.

Hoofdstuk 2 geeft een overzicht van klinische en therapeutische aspecten van syfilis.

Hoofdstuk 3 geeft, op literatuurgegevens gebaseerd, een overzicht betreffende serologische syfilisreacties. Hierbij wordt uitgegaan van de verdeling van deze reacties in non-treponemale en treponemale reacties. Deze verdeling is gebaseerd op de herkomst van het bij deze reacties toegepaste antigeen. Vooral wordt ingegaan op de verschillende reacties die in Nederland verricht worden. Daarnaast worden ook nog de begrippen anti-complementariteit en biologisch vals-positieve reacties besproken.

In hoofdstuk 4 worden de specifieke vraagstellingen voor het onderzoek geformuleerd:

1) hoe is het beloop van een aantal non-treponemale en treponemale serologische reacties, zowel bij het onbehandeld doormaken van een venerisch verworven infectie met T. pallidum als na een behandeling met penicilline, en zijn er daarbij nog verschillen na behandeling volgens twee verschillende behandelingsschema's ?
2) kunnen een tweetal in Nederland veel gebruikte combinaties van reacties als effectief bij de opsporing van syfilis worden beschouwd en in welke mate zou de TPHA hieraan kunnen bijdragen ?

In hoofdstuk 5 worden achtereenvolgens de toegepaste diagnostische criteria, serologische technieken, behandeling, computerbewerking, statistische methoden, wijze van grafische weergave en nacontrole van behandelde patienten besproken.

In hoofdstuk 6 wordt het beloop van een aantal non-treponemale en treponemale serologische reacties bij het zonder behandeling voortbestaan van een infectie met T. pallidum beschreven. De grafische weergave hiervan is gebaseerd op de mediaan van de waarnemingen bij de verschillende

groepen vóór behandeling. Tevens is het percentage positieve reacties (sero-reactiviteit) voor de verschillende reacties in verschillende ziektestadia berekend. Bij primaire syfilis blijkt dat de VDRL significant vaker positief is dan de Kolmer en bestaat de volgende volgorde van afnemende gevoeligheid:
FTA-ABS ≃ RPR > RPCF ≃ VDRL > Kolmer ≃ TPI.
FTA-ABS en RPR zijn het gevoeligst, het verschil in gevoeligheid tussen deze twee reacties was niet significant.
Bij laat latente syfilis is deze volgorde als volgt:
TPI ≃ FTA-ABS > RPCF ≃ RPR > VDRL ≃ Kolmer.
Uit de grafische weergave blijken grote verschillen in beloop van de diverse serologische syfilisreacties. Met name is het verschil duidelijk tussen het beloop van de non-treponemale reacties (Kolmer, VDRL en RPR) en de type-specifieke treponemale reacties (FTA-ABS en TPI). Gewezen wordt op het vóórkomen van hoge titers in non-treponemale reacties bij patienten met symptomatische neurosyfilis. Er worden argumenten genoemd die het belang van herhaald verrichten van serologisch bloedonderzoek bij verdenking op syfilis aangeven.

In hoofdstuk 7 wordt een grafische weergave gegeven van het beloop van Kolmer, VDRL, RPCF, FTA-ABS en TPI gedurende 2-5 jaar na behandeling met penicilline. Gewezen wordt op de grote verschillen die ook hierbij bestaan tussen non-treponemale en treponemale reacties bij patienten met vroege infectieuze syfilis. Bij laat latente syfilis blijkt dat behandeling met penicilline geen invloed heeft op het beloop van de reacties gedurende een observatieperiode van vier tot vijf jaar. Naarmate de ziekte langer heeft bestaan vindt afname van de reactiviteit van de verschillende reacties ook later, of zelfs in het geheel niet plaats. Een exacte classificatie van de ziekte vóór behandeling en het rekening houden met het tijdstip van behandeling zijn van groot belang bij het beoordelen van resultaten van serologische syfilisreacties. Er blijken, bij niet-gerandomiseerd onderzoek, geen significante verschillen te bestaan tussen het beloop van serologische syfilisreacties na behandeling met minstens 31,2 miljoen I E penicilline gedurende minstens 6 weken en 10,8 miljoen I E penicilline gedurende 6 weken.

In hoofdstuk 8 is nagegaan of enkele veel in Nederland gebruikte combinaties van reacties, met name VDRL/RPCF en VDRL/Kolmer effectief zijn voor aantonen of uitsluiten van syfilis. In tabelvorm zijn hierbij nog enkele

belangrijke eigenschappen van de verschillende reacties genoemd. Tevens is de TPHA, die thans eveneens in een aantal laboratoria verricht wordt, bij het onderzoek betrokken. Het blijkt dat met het tweetal bovengenoemde combinaties van reacties de diagnose syfilis bij 20,3% resp. 37,2% van de bij dit onderzoek betrokken syfilispatienten gemist zou zijn. Getracht wordt om het kiezen van, voor screening te verrichten, serologische reacties te vergemakkelijken door het berekenen van de predictieve waarde van positieve test-resultaten. Daarnaast is vooral bij bloeddonoren van belang te weten hoe groot de kans is dat een donor bij wie een negatieve reactie is gevonden, toch aan syfilis lijdt. Geconcludeerd wordt dat deze laatste kans het kleinst is bij de TPHA en dat daarom deze reactie voor keuring van bloeddonoren kan worden aanbevolen. Bij serologisch onderzoek voor het aantonen van syfilis bij andere groepen wordt gebruik van de TPHA in combinatie met een non-treponemale reactie, bij voorkeur de RPR geadviseerd.
Ook wordt gewezen op het belangrijke voordeel van een uniformiteit van door de streeklaboratoria te verrichten reacties, waardoor een mogelijke bron van verwarring en misverstanden kan verdwijnen.

REFERENCES

d'Alessandro, G. et al. The antigens of the cultured Treponema pallidum and the antispirochetal antibodies in human syphilis. J Ven Dis Inf 31: 314–315, 1950.

Atwood, W. G. et al. The TPI and FTA-ABS tests in treated late syphilis. JAMA 203: 549–551, 1968.

Bänffer, J. R. J. et al. Evaluation of the counter-immunoelectrophoresis technique in syphilis serology. Br J Vener Dis 50: 101–103, 1974.

Bännfer, J. R. J. et al. Comparison of the Counter-Immuno-Electrophoresis technique with the Reiter Protein and three other serological tests as a first line test for syphilis. J Clin Microb 2: 362–367, 1975.

Barniske, R. Erfahrungen mit dem Nelson-test an der Hautklinik der Joh. Gutenberg-Universität Mainz. Z Haut u Geschlkr 23: 290–302, 1957.

Barton, R. L. et al. Treatment of early syphilis with penicillin. Arch Dermatol 60: 150–154, 1949.

Bauer, T. J. et al. The treatment of early syphilis with 600.000 units of penicillin in seven and one half days. Am J Syph 31: 45–48, 1947.

Bauer, T. J. Evaluation of antisyphilitic therapy with intensive follow-up. I. The plan. J Ven Dis Inf 3: 355–379, 1951.

Bekker, J. H. and J. H. de Bruyn. Nieuwe methoden bij de serologische syphilisdiagnostiek. Ned T Geneesk 97: 3286–3289, 1953.

Bekker, J. H. and P. C. Onvlee. De waarde van de Treponema-pallidum-immobilisatie-reactie voor de diagnostiek van syphilis. Ned T Geneesk 99: 1414–1421, 1955.

Bekker, J. H. De klinische interpretatie van de moderne serologische reacties bij de diagnostiek van syfilis. Ned T Geneesk 104: 1935–1937, 1960.

Black, D. A. et al. Qualitative evaluation of the reagin screen test. J Clin Microb 4: 16–18, 1976.

Blum, G. et al. Reliability of the Treponemal Haemogglutanation Test for the serodiagnosis of syphilis. J Infect Dis 127: 321–325, 1973.

Bolle, E. A. W. et al. Beschrijvende statistiek. 1st ed., Kluwer, Deventer, 1973.

Bos, J. D. et al.[a] Antitreponemal IgE in early syphilis. Br J Vener Dis 56: 20–25, 1980.

Bos, J. D. et al.[b] T-Lymphoid cells in primary syphilis. Br J Vener Dis 56: 74–76, 1980.

Brown, W. J. et al. Evaluation of RPR Card Test for syphilis screening in field investigations. Public Health Rep 79: 496–500, 1964.

Bruusgaard, E. Über das Schicksal der nicht specifisch behandelten Luetiker. Arch Dermat Syph (Berlin) 157: 309–332, 1929.

de Bruyn, J. H. A standardized routine complement fixation test. Antonie van Leeuwenhoek 24: 69–75, 1957.

de Bruyn, J.H. and J.H. Bekker. De toepassing van een eiwitantigeen van Treponema pallidum (reiter-stam) bij de complementbindingsreactie. Ned T Geneesk 101: 1615-1617, 1957.

Buchanan, G.S. et al. FTA-ABS test in pregnancy. Arch Dermatol 102: 322-325, 1970.

Burns, R.E. Spontaneous reversion of FTA-ABS test reactions. JAMA 234: 617-618, 1975.

Cannefax, G.R. et al. Reiter Protein Complement Fixation Test for syphilis. Public Health Rep 72: 335-340, 1957.

Capinsky, T.A. et al. Antibiotics in the treatment of early syphilis in 'Antibiotic treatment of venereal diseases' (Luger, A., ed.) p. 41-45, Karger, Basel, 1968.

Carpenter, C.M. et al. A triple test plan for the serologic diagnosis of syphilis. N Engl J Med 263: 1016-1018, 1960.

Catterall, R.D. Systemic disease and the biological false positive reaction. Br J Vener Dis 48: 1-12, 1972.

Cave, V.G. The management of the several stages of syphilis. J Nat Med Assoc 67: 289-293, 1975.

Chester, B.J. et al. Serologic observations following penecillin treatment for latent syphilis. Am J Syph 37: 7-17, 1953.

Collart, P. et al. Persistance du tréponème pale après traitement. Ann Inst Pasteur (Paris) 102: 693-704, 1962.

Collart, P. et al. Significance of spiral organisms found, after treatment, in late human and experimental syphilis. Br J Vener Dis 40: 81-89, 1964.

Collart, P. Critique des éléments de base sur lesquels reposent certains traitements de la syphilis. Sem Hop Paris 50: 673-679, 1974.

Crampon, P. and J. Baelden. Le test de Nelson. Ann Inst Past (Lille) 5: 191-201, 1952.

Csonka, G.W. Luotest, a preliminary evaluation in the diagnosis of late syphilis. Medicine Illustrated 4: 389-391, 1950.

Cutler, J.C. et al. A report on an observed pattern of entrance into sero-reactivity among patients with untreated primary syphilis. J Ven Dis Inf 33: 533-544, 1952.

Dandoy, S. Initial serological reactions in infectious syphilis. Br J Vener Dis 43: 105-110, 1967.

Dans, P.E. et al. The FTA-ABS test: a diagnostic help or hindrance? South Med J 70: 312-315, 1977.

Deacon, W.E. et al. A fluorescent test for treponemal antibodies. Proc Soc Exp Biol Med 96: 477-480, 1957.

Deacon, W.E. and E.F. Hunter. Treponemal antigens as related to identification and syphilis serology. Proc Soc Exp Biol Med 110: 352-356, 1962.

Deacon, W.E. et al. Fluorescent Treponemal Antibody-Absorption (FTA-ABS) test for syphilis. JAMA 198: 624-628, 1966.

Degos, R. Syphilis et la grossesse. Rev Franc Gynaec 66: 551-555, 1971.

Doniach, D. Autoantibodies in syphilis and in chronic biological false positive reactions. In Sexually Transmitted Diseases. Ed R. D. Catterall and C. S. Nicol. Acad. Press, London, 1976.

Duncan, W. C. et al. The FTA-ABS Test in dark-field positive primary syphilis. JAMA 228: 859-860, 1974.

Dunlop, E. M. C. et al. Penicillin levels in blood and CSF achieved by treatment of syphilis. JAMA 241: 2538-2540, 1979.

Durel, P. et al. Propositions pour un diagnostic sérologique moderne de la syphilis. Bull Soc Franc Derm Syph 72: 501-514, 1965.

Durst, R. D. et al. Dose-related seroreversal in syphilis. Arch Dermatol 108: 663-664, 1973.

Dyckman, J. D. et al. Evaluation of Reagin Screen Test, a new serological test for syphilis. J Clin Microb 4: 145-150, 1976.

Dzink, P. E. et al. Syphla-Chek: a qualitative study. J Clin Microb 5: 593-595, 1977.

Eagle, H. and R. B. Hogan. On the presence in syphilitic serum of antibodies to spirochetes, their relation to so called Wassermann reagin and their significance for the serodiagnosis of syphilis. J Exp Med 71: 215-230, 1940.

Editorial: Persistence of treponemes after treatment of syphilis. Lancet II: 718, 1968.

Edmundson, W. F. et al. Study of the TPI test in clinical syphilis. Arch Dermatol 71: 387-390, 1955.

Ehrke, K. et al. Treponema-Pallidum-Hämagglutinations (TPHA)-Test. Beurteilung der Leistungsfähigkeit als Lues-screening bei Blutspendern. Arztl Lab 23: 425-430, 1977.

Ehrmann, G. Uber die provozierbarkeit des TPI-tests Nelson-Mayer und eine Möglichkeit der therapeutischen Beeinflussung der sog. seroresistenten Lues. Arch Klin Exp Derm 227: 993-1004, 1967.

Eng, J. and K. Wereide. TPI test in untreated syphilis. Br J Vener Dis 38: 223-229, 1962.

Falcone, V. H. et al. Evaluation of Rapid Plasma Reagin (Circle) Card test. Public Health Rep 79: 491-495, 1964.

Ferro, H. Grafiekenpapier. Statistica 8: 123-154, 1954.

Fiumara, N. J. The treatment of syphilis. N Engl J Med 270: 1185-1188, 1964.

Fiumara, N. J. Treatment of seropositive primary syphilis: an evaluation of 196 patients. Sex Transm Dis 4: 92-95, 1977.

Fiumara, N. J. Treatment of secondary syphilis: an evaluation of 204 patients. Sex Transm Dis 4: 96-99, 1977.

Fiumara, N. J. Reinfection primary and secondary syphilis. The post treatment serologic response. Sex Transm Dis 4: 132-134, 1977.

Fiumara, N. J. Treatment of early latent syphilis of less than a year's duration. Sex Transm Dis 5: 85-88, 1978.

Förström, L. Reiter Protein Complement Fixation Test as a serological test for syphilis. Acta Derm Venereol (Suppl 59) 47, 1967.

From, E. et al. Reactivity of lymphocytes from patients with syphilis towards T-pallidum antigen in the leucocyte migration and lymphocyte transformation tests. Br J Vener Dis 52: 224-229, 1976.

Gaehtgens, W. Uber die antigene Wirkung von Pallidasuspensionen in carbolisierter Kochsalzlösung. Med Klin 25: 390-392, 1929.

Gahlen, W. Die Grenzen des Normalen beim Rückgang der Seroreaktionen nach Luesbehandlung. Hautartzt 4: 380-384, 1953.

Gahlen, W. and F. Ninneman. Versuch einer varianzanalytischen Synopsis der Lues-serologischen Ergebnisse nach Penicillin-Behandlung. Hautartzt 16: 300-304, 1965.

Garner, M.F. et al. The Treponema pallidum haemagglutination (TPHA) test in biological false positive and leprosy sera. J Clin Path 26: 258-260, 1973.

Ghinsberg, R. et al. Specificity and sensitivity of the T.pallidum haemagglutination test in syphilitic and non-syphilitic sera. WHO/VDT/RES/ 72: 289, 1972.

Gjestland, T. Oslo study of untreated syphilis. Acta Derm Venereol (suppl 34) 35: 1-368, 1955.

Grabner, K. Zwischenbericht über die Erfahrungen des Arbeitskreises 'Therapie der Syphilis'. Z Haut u Geschl kr 44: 849-856, 1969.

Green, R.L. et al. Increased immunoglobulin E concentrations in venereal diseases. Br J Vener Dis 52: 257-260, 1976.

Guthe, T. and O. Idsoe. Antibiotic treatment of syphilis in 'Antibiotic treatment of venereal diseases' (Luger, A., ed.) p. 2-33, Karger, Basel, 1968.

Hagedorn, H.J. and P. Naumann. Moderne Serodiagnostik der syphilis. Dtsch Med Wochenschr 104: 209-214, 1979.

Harner, R.E. et al. The FTA-ABS test in late syphilis. JAMA 203: 545-548, 1968.

Harris, A. et al. A microflocculation test for syphilis using cardiolipin antigen. J Ven Dis Inf 27: 169-174, 1946.

Hatos, G. Evaluation of 460 cases of treated syphilis. Med J Aust 2: 415-420, 1972.

Hederstedt, B. et al. Quantitative Treponema pallidum immobilization (TPI) test in early syphilis. Acta Derm Venereol 44: 82-90, 1964.

Hoekenga, M.T. et al. The relationship of early clinical failure to serologic response in penicillin-treated early syphilis. Am J Syph 33: 515-522, 1949.

Hughes, K.M. et al. Positive fluorescent treponemal antibody reactions in diabetes. Appl Microbiol 19: 425-428, 1970.

Hunter, E.F. et al. An improved FTA test for syphilis, the absorption procedure (FTA-ABS). Public Health Rep 79: 410-412, 1964.

Hunter, E.F. et al. The fluorescent treponemal antibody-absorption (FTA-ABS) test: development, use and present status. Bull WHO 39: 873-881, 1968.

Hunter, E.F. The fluorescent treponemal antibody-absorption (FTA-ABS) test for syphilis. CRC Crit Rev Clin Lab Sci 5: 315-330, 1975.

Huriez, C. and J. Dujardin. La pénicillotherapie des syphilis tardive ou compliquees. Press Med 57: 1-2 1949.

Huriez, C. et al. Exploration immuno-allergologique dans la syphilis tardive. Lille Med 6: 178-182, 1961.

Idsoe, O. et al. Penicillin in the treatment of syphilis. Bull WHO Suppl to vol 147, 1972.

Iskrant, A.P. et al. III Statistical method of analysis and its critical evaluation. J Ven Dis Inf 32: 371-375, 1951.

Jaeger, H. and J. Delacrétaz. Importance du test d'immobilisation des tréponèmes dans le séro-diagnostic de la syphilis à ses différents stades. Dermatologica 106: 256-263, 1953.

Johannson, E.A. The use of standard serological tests for syphilis in screening for auto-immune connective tissue disease. Acta Derm Venereol 50: 305-308, 1970.

Jong, N.H.J. de, et al. Epidermal antibodies in secondary syphilis. Correspondence. Br J Vener Dis 54: 283, 1978.

Kaufman, R.E. et al. The FTA-ABS (IgM) test for neonatal congenital syphilis: a critical review. J Am Vener Dis Ass 1: 79-83, 1974.

Kent, J.F. and J.B. de Weerdt. Enhancement by lysozyme of the sensitivity of Treponema Pallidum Immobilization tests. Br J Vener Dis 39: 37-40, 1963.

Kerl, H. et al. Beurteilung und Klinische Bedeutung des Treponema-Pallidum-Haem-Agglutinations (TPHA)-Testes in der Syphilisdiagnostik. Z Hautkr 51: 718-726, 1976.

Kiraly, K. and H. Prerau. Evaluation of the T. pallidum haemagglutination (TPHA) test for syphilis on 'problem sera'. Acta Derm Venereol 54: 303-310, 1974.

Knox, J.M. et al. The FTA-ABS test for syphilis. Br J Vener Dis 42: 16-20, 1966.

Kolmer, J.A. Studies in the standardization of the Wassermann reaction. Am J Syph 6: 82-110, 1922.

Kolmer, J.A. Cardiolipin Antigens in the Kolmer Complement Fixation Test for syphilis. J Ven Dis Inf 29: 166-172, 1948.

Koning, G.A.J. de, et al. A patient with an extra-genital luetic primary lesion of the hand. Br J Vener Dis 53: 386-388, 1977.

Landsteiner, K. et al. Zur Frage der Komplement-bindungsreaktionen bei Syphilis. Wien Klin Wochenschr 20: 1565-1567, 1907.

Lassus, A. et al. The order of appearance of reactivity to treponemal and lipoidal tests in early syphilis. Acta Pathol Microbiol Scand 69: 612-613, 1976.

Lassus, A. and K.K. Mustakallio. Anticomplementary activity in serological tests for syphilis as a clue to connective tissue diseases of an autoimmune nature. Ann Clin Res 1: 74-76, 1969.

League of Nations. Quart Bull Hlth Org L.o.N. 4: 129-246, 1935.

Lesinsky, J. et al. Specificity, sensitivity and diagnostic value of the TPHA test. Br J Vener Dis 50: 334-340, 1974.

Levene, G.M. et et. Cell-mediated immunity and lymphocyte transformation in syphilis. Proc R Soc Med 64: 426-429, 1971.

Lindemayr, W. and W. Partsch. Kongenitale syphilis. Z Hautkr 51: 749-756, 1976.

Luger, A. Antibiotic treatment of venereal diseases. Karger, Basel, 1968.

Luger, A. and I. Spendlingwimmer. Der Automatisierte Mikro-Haemagglutinations-test mit Treponema Pallidum Antigen (AMHA-TP-test). Hautartzt 25: 238-244, 1974.

Luger, A. et al. Das Verhalten der Reaktivität in syphilis-serologische Untersuchungsmethoden. Z Hautkr 49: 529-540, 1974.

Luger, A. et al. Quantitative evaluation of the FTA-ABS-IgM test in treated and untreated syphilis. Br J Vener Dis 53: 287-291, 1977.

Luger, A. Das Problem der persistierenden Treponemen. Hautartzt 31: 237-244, 1980.

Magnuson, H.J. et al. Treponemal Immobilization Test of normal and syphilitic serums. J Ven Dis Inf 1, 309-320, 1949.

Magnuson, H.J. et al. Inoculation syphilis in human volunteers. Medicine (Baltimore) 35: 33-81, 1956.

Magnuson, H.J. and Ch.P. McLeod. Treponema pallidum agglutination tests. Bull WHO 14: 289-302, 1956.

Mahoney, J.F. Penicillin treatment of early syphilis. A preliminary report. J Ven Dis Inf 24: 355-357, 1943.

Malloy, A.M. and R.L. Kahn. The ultramicroscopic precipitation reaction in syphilis. J Infect Dis 48: 243-254, 1931.

Manual of Tests for Syphilis. U.S. Department of HEW, publication no (CDC) 77-8347, Atlanta, 1969.

Menke, H.E. Immuunglobulinen en thymoltroebeling bij vroege syphilis. Thesis, Erasmus University Rotterdam, 1975.

Menke, H.E. et al. Comparison of cardiolipin and treponemal tests in the serodiagnosis of yaws. Br J Vener Dis 55: 102-104, 1979.

Menke, H.E. and A. Notowicz. Treponema pallidum haemagglutinatie test (TPHA), to be published, 1981.

van de Merwe, J.P. Betekenis van biologisch fout positieve luesreacties. Nascholingscursus Ned Ver v Dermatologen, Eindhoven, Abstract p 59, 1975.

Miller, J.L. et al. Studies with the treponemal immobilizing test. JAMA 154: 1241-1247, 1954.

Mohr, J.A. et al. Neurosyphilis and penicillin levels in cerebrospinal fluid. JAMA 236: 2208-2209, 1976.

Moore, J.E. The diagnosis of syphilis by the general practitioner. J Ven Dis Inf suppl 23, 1949.

Moore, J.E. et al. The problem of seroresistant syphilis. JAMA 110: 96-100, 1938.

Moore, J. E. and C. F. Mohr. Biologically false positive serologic tests for syphilis. JAMA 150: 467–473, 1952.

Moore, M. B. et al. Sensitivity and specificity in syphilis serology: clinical implications. South Med J 58: 963–968, 1965.

Müller, F. Zur Technik des Nachweises treponemen-spezifischer 19S-IgM-antikörper bei der latenten und spätlatenten Syphilis. Immunität und Infektion 5: 109–113, 1977.

Mustakallio, K. K. et al. Auto-immune phenomena in syphilitic infection: rheumatoid factor and cryoglobulins in different stages of syphilis. Int Arch Allergy Appl Immunol 31: 417–426, 1967.

Nelson, R. A. and H. G. Steinman. Factors affecting the survival of Treponema pallidum in vitro. Proc Soc Exp Biol Med 68: 588, 1948.

Nelson, R. A. and M. M. Mayer. Immobilization of Treponema pallidum in vitro by antibody produced in syphilitic infection. J Exp Med 89: 369–392, 1949.

Nielsen, H. A. and A. Reyn. The Treponema pallidum immobilization test. Bull WHO 14: 263–288, 1956.

Niemel, P. L. A. et al. Attenuated yaws in Surinam. Br J Vener Dis 55: 99–101, 1979.

Notowicz, A. and H. E. Menke. Atypical primary syphilic lesions on the penis. Dermatologica 147: 328–333, 1973.

Notowicz, A. et al. Solitary papular lesions on the penis in insufficient treated early syphilis. Dermatologica 150: 26–31, 1975.

Obeid-Ruggli, V. M. E. Uber Resultate der Penicillin-behandlung der Syphilis mit einer protrahierten Kur von 14.4 Millionen Einheiten. Schweiz Med Wochenschr 90: 820–826, 1960.

Olansky, S. et al. Immune-adherence test for syphilis. Public Health Rep 69: 521–526, 1954.

Olansky, S. et al. Untreated syphilis in the male negro. Arch Dermatol 73: 516–522, 1956.

Olansky, S. and L. C. Norins. Current serodiagnosis and treatment of syphilis. JAMA 198: 165–168, 1966.

O'Neill, P. et al. Treponema pallidum haemagglutination assay in the routine serodiagnosis of treponemal disease. Br J Vener Dis 49: 427–431, 1973.

Ovcinnikov, N. M. and G. F. Timcenko. The haemagglutination test (TPHA) in the serodiagnosis of syphilis. WHO/VDT/RES 74: 315, 1974.

Parent, M. A. and P. M. Smythe. Dinitrichlorobenzene sensitation in congenital syphilis. Lancet II: 1273, 1973.

Pepplinkhuizen, L. and H. E. Menke. Het syndroom van Hoigné. Ned T Geneesk 121: 609–612, 1977.

Perdrup, A. Penicillin treatment of early syphilis. Acta Derm Venereol 40: 340–357, 1960.

Perrot, H. et al. Cryoglobuline et facteur rhumatoide au cours de la syphilis primosecondaire. Presse Medicale 7: 1059–1060, 1971.

Plotke, F. et al. The total dosage factor in the use of crystalline penicillin G. Am J Syph 35: 240–245, 1951.

Portnoy, J. and H. J. Magnuson. Treponema pallidum complement fixation (TPCF) test for syphilis. Am J Clin Pathol 26: 313–322, 1956.

Portnoy, J. et al. Rapid Plasma Reagin Card Test for syphilis and other Treponematoses. Public Health Rep 77: 645–652, 1962.

Rathlev, T. Hemagglutination tests utilizing antigens from pathogenic and apathogenic Treponema pallidum WHO/VDT/RES/77: 65, 1965.

Reimer, C. B. et al. The specificity of fetal IgM: antibody or anti-antibody? Ann NY Acad Sci 254: 77–93, 1975.

Rein, C. R. The serologic tests in penicillin-treated syphilis. NY State J Med 47: 2450–2452, 1947.

Ritter, G. et al. Blut-Liquor-Kinetik von Penicillin G bei Neurosyphilis. Munch Med Wochenschr 117: 1383–1386, 1975.

Rockwell, D. H. et al. The Tuskegee Study of untreated syphilis. Arch Intern Med 114: 792–798, 1964.

Rosahn, P. D. and C. L. Rowe. Experimental mouse syphilis II, minimal infectious number of Treponema pallidum. Am J Syph 34: 40–44, 1950.

Salo, O. P. et al. Quantitative Reiter Protein Complement-Fixation Test. Br J Vener Dis 43: 264–266, 1967.

Schmidt, B. The 17S-IgM-FTA-ABS Test in the serum diagnosis of syphilis. WHO/VDT/RES 79: 362, 1979.

Schuller, J. C. Anticomplementaire, chronisch biologisch vals positieve en seroresistente luesreacties. Ned T Geneesk 117: 1914, 1973.

Schroeter, A. L. Treatment for early syphilis and reactivity of serologic tests. JAMA 221: 471–476, 1972.

Scotti, T. A. and L. Logan. A specific IgM antibody test in neonatal congenital syphilis. J Pediatr 73: 242–243, 1968.

Sequeira, P. J. L. An examination of the Treponemal Wassermann Reaction and Reiter Protein Complement-Fixation Test. Br J Vener Dis 35: 139–147, 1959.

Sequeira, P. J. L. and A. E. Eldridge. Treponemal haemagglutination test. Br J Vener Dis 49: 242–248, 1973.

SERA – Serology evaluation and research assembly, U. S. Public Health Service: 1956–1957, results abstracted in Förström, L. (1967).

Shafer, J. K. et al. Long term studies of results of penicillin therapy in early syphilis. Bull WHO 10: 563–578, 1954.

Shannon, R. and S. D. Booth. The pattern of immunological responses at various stages of syphilis. Br J Vener Dis 53: 281–286, 1977.

Smithurst, B. E. Penicillin therapy in 44 cases of primary and secondary syphilis. Med J Aust 1: 248–250, 1971.

Spendlingwimmer, I. Der TPHA-test, eine neue Methode zum Nachweis einer Syphilisinfektion. Z Hautkr 51: 788–790, 1976.

Steinmann, W. Das post-therapeutische Verhalten der Komplementbindungsreaktion nach Wassermann-Neisser-Bruck. Thesis. Aachen, 1976.

Stokes, J.H. et al. Modern Clinical Syphilology, 3rd ed., Saunders, Philadelphia, 1941.

Stolz, E. Diagnostic aspects of gonorrhoea. Thesis. Erasmus University Rotterdam, 1974.

Stout, G.W. et al. Preparation and standardization of the sorbent used in the fluorescent treponemal antibody-absorption (FTA-ABS) test. Health Lab Sci 4: 5-8, 1967.

Stütgen, G. and J. Bartunek. Zur Kurzbehandlung der Frühlues mit maximal dosierten Penicillin-Infusionen. Med Welt 24: 219-221, 1973.

Thompson, S.E. et al. Why review syphilis therapy now? J Am Vener Dis Assoc 3: 98, 1976.

Tio, B.S. Comparison of the Brewer rapid plasma reagin card test with other tests for syphilis. Br J Vener Dis 46: 287-289, 1970.

Tomizawa, T. and S. Kasamatsu. Hemagglutination tests for diagnosis of syphilis. A preliminary report. Jpn J Med Sci Biol 19: 305-308, 1966.

Tomizawa, T.S. et al. Usefullness of Hemagglutination Test using Treponema pallidum Antigen (TPHA) for serodiagnosis of syphilis. Jpn J Med Sci Biol 22: 341-350, 1969.

Tramont, E.C. Persistence of Treponema pallidum following penicillin G therapy. JAMA 236: 2206-2207, 1976.

Valkenburg, H.A. Reumafactortest. Ned T Geneesk 118: 413-421, 1974.

Vecchio, Th.J. Predictive value of a single diagnostic test in unselected populations. N Engl J Med 274: 1171-1173, 1966.

Verlaeckt, H. and J. Verstraete. Serologische syfilisdiagnostiek. Een vergelijkende studie van vijf verschillende tests uitgevoerd op 3500 sera. Tijdschr Geneesk 23: 1343-1353, 1976.

Veldkamp, J. and A.M. Visser. Application of the enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of syphilis. Br J Vener Dis 51: 227-231, 1975.

Vliet, A.G.M. van. Neurolues nieuwe stijl. Ned T Geneesk 117: 1913, 1973.

Vonderlehr, R.A. et al. Untreated syphilis in the male negro. J Ven Dis Inf 17: 260-265, 1936.

Wassermann, A. et al. Eine serodiagnostische Reaktion bei syphilis. Dtsch Med Wochenschr 32: 745-746, 1906.

Whartin, A.S. and A.C. Starry. Second improved method for the demonstration of spirochaeta pallida in the tissues. JAMA 76: 234-237, 1921.

Wiedmann, A. 20 Jahre Penicillinbehandlung der Kongenitalen Syphilis. Arch Klin Exp Derm 219: 193, 1964.

Wigfield, A.S. Immunological phenomena of syphilis. Br J Vener Dis 41: 275-285, 1965.

Wilkinson, A.E. Some aspects of research on syphilis: serological evidence of activity of the disease. In Sexually Transmitted Diseases: 214-218. Ed. R.D. Catterall and C.S. Nicol. Acad. Press, London, 1976.

Wilkinson, A.E. and L.P. Cowell. Immunofluorescent staining for the detection of treponema pallidum in early syphilitic lesion. Br J Vener Dis 47: 252-254, 1971.

Wilkinson, A.E. and P. Rodin. IgM-FTA test in syphilis in adults-Its relation to clinical findings. Br J Vener Dis 52: 219-223, 1976.

Wood, R.M. et al. Comparison of the Fluorescent Treponema Antibody and immobilization tests on serums from 1182 diagnostic problem cases. Am J Clin Path 47: 521-524, 1967.

Wright, J.T. et al. False positive FTA-ABS results in patients with genital herpes. Br J Vener Dis 51: 329-330, 1975.

Yoder, F.W. Penicillin treatment of neurosyphilis. JAMA 232: 270-271, 1975.

Young, H. et al. Treponema pallidum haemagglutination test as a screening procedure for the diagnosis of syphilis. Br J Vener Dis 50: 341-346, 1974.

DANKWOORD

Dit proefschrift werd bewerkt op de afdeling Dermato-Venereologie van het Academisch Ziekenhuis Dijkzigt en de Medische Faculteit van de Erasmus Universiteit te Rotterdam.
De aanzet voor dit onderzoek werd gegeven door Prof. Dr. C. H. Beek, aan wie ik veel dank verschuldigd ben. Hij gaf mij de kans mijn klinische werkzaamheden te combineren met dit onderzoek. Zijn opvolger, Prof. Dr. E. Stolz, gaf mij alle mogelijkheden en hulp om het onderzoek voort te zetten. Vele gesprekken met hem hebben de uiteindelijke vorm bepaald.
Henk Menke en Jaap van der Sluis hebben veel tijd besteed aan het kritisch doorlezen tijdens verschillende fasen van het bewerken van het materiaal.
De co-referenten Prof. Dr. M. F. Michel en Prof. Dr. H. A. Valkenburg hebben, in een later stadium, waardevolle adviezen gegeven.
De medewerking van de arts-assistenten, die mij als Chef de Clinique tijdens hun opleidingstijd in de kliniek nogal eens hebben moeten missen, heb ik zeer gewaardeerd.
De serologische reacties, waarvan de resultaten de basis voor dit onderzoek vormden, werden verricht door de dames G. J. Tideman-Valkenburgh en A. M. Visser en de heren G. N. M. Aalbers, J. C. Compeer en P. C. Onvlee (RIV-Bilthoven) en de dames G. Bosscher-Koetsier, M. de Jonge-Suij, S. de Weerdt-van Ameiden en H. Zwart (Academisch Ziekenhuis Rotterdam).
De heer R. de Haan (afd. Automatische Signaal Verwerking, Erasmus Universiteit) heeft met veel geduld, de vaak herhaalde computerbewerkingen uitgevoerd.
Drs. H. J. A. Schouten (afd. Biostatistica, Erasmus Universiteit) adviseerde bij alle statistische bewerkingen.
Magda de Ridder-Goetjaer en Johan van der Stek hebben, ieder op hun gebied, gewaardeerde bijdragen geleverd.
De heer Th. van Winsen verzorgde de Engelse vertaling.
Het meeste typewerk, inclusief het definitieve manuscript, werd verricht door mijn echtgenote Jacqueline.
De vele uren die ik, naast mijn normale werktijd, buitenshuis heb doorgebracht met dit onderzoek, hebben mijn kinderen Ajal en Liora een afkeer van wetenschappelijk werk bijgebracht. Hopenlijk is dit slechts tijdelijk.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 18 november 1945 te Amsterdam geboren. Na het behalen van het diploma HBS-B studeerde hij van 1962 tot 1970 geneeskunde aan de Rijksuniversiteit Leiden. Na de vervulling van de militaire dienstplicht en kortdurende werkzaamheden bij de GGD Rotterdam, begon hij op 10 april 1972 de specialisatie tot dermato-venereoloog op de afdeling Dermato-Venereologie van het Academisch Ziekenhuis Rotterdam (opleider: Prof. Dr. C. H. Beek). Na inschrijving in het Specialistenregister op 10 april 1976, bleef hij, eerst als hoofdgeneeskundige, daarna als wetenschappelijk hoofdmedewerker werkzaam op eerdergenoemde afdeling, vanaf 1 januari 1978 onder leiding van Prof. Dr. E. Stolz. Van 1 september 1975 tot 1 september 1980 fungeerde hij als Chef de Clinique. Vanaf 1 augustus 1979 is hij parttime bij deze afdeling werkzaam. Daarnaast is hij gevestigd als dermato-venereoloog in het Westeinde Ziekenhuis te Den Haag.