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Erasmus Journal of Medicine: independent scientific journal

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Original contribution

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Colofon

Erasmus Journal of Medicine is a scientific magazine by and for students of Erasmus MC University Medical Center Rotterdam. It was initiated by the MFVR (the students' organization of Erasmus MC).
The journal will appear twice a year. It will be published on paper (2000 copies) and on the EJM website (www.erasmusjournalofmedicine.nl).
The main purpose of the journal is to stimulate Erasmus MC medical students to read and write about medical scientific subjects, early in their career. A secondary purpose is to make the results of excellent student-driven research known to others. The journal contains papers describing original research (Full articles), systematic reviews (Reviews), summaries of recently conducted studies (Extended abstracts), short descriptions of research projects looking for students to participate (Research News), opinion papers written by students (Opinions), editorial comments and letters to the editor.
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Foreword

Dear Reader,

The first issue of the Erasmus Journal of Medicine, published in May 2010, was a great success in every respect. Not only was the quality of the scientific articles high, so was the enthusiasm and willingness with which our medical students and lecturers submitted their contributions. The editorial quality and the design of our own scientific journal are also in line with what may be expected of a scientific journal.

Erasmus Journal of Medicine has passed an important first test. The editorial team has succeeded in making a high quality journal with its own image, with readable content and with an interested and involved group of authors and readers. The challenge is now to continue and proceed on this chosen path. One issue does not make a journal yet. It is up to us, as academic community of Erasmus MC, to deliver content and commitment over the coming years which can result in a new, valuable tradition supporting the scientific development of our medical students and the identity and image of our university medical center.

Over the past months, students, lecturers and educational experts from Desiderius School, who play an important role in supporting the education, have worked hard on the future outlook of medical education in Rotterdam. They all agree that scientific development is essential, a conditio sine qua non. It goes without saying that a physician has to keep up with scientific developments and is capable of satisfactorily selecting and interpreting new information relevant to his or her discipline. Erasmus Journal of Medicine is consistent with this view on medical education. It offers a complete platform for the first steps in a scientific career.

Therefore, I sincerely encourage students to publish their research in the columns of this magnificent journal. Naturally, I would also like to take this opportunity to wish all readers, both inside and outside Erasmus MC, happy reading and I hope you enjoy this second issue.

Huibert Pols, Dean

‘Great learning experience’

The success of the first issue of Erasmus Journal of Medicine proves that students need a medium to publish their work and share ideas with their peers. The overall objective of EJM is to publish scientific articles written by students.

However, the Editorial Board of EJM has several other objectives that are at least as important as publishing articles. To let the medical students get acquainted with the broad palette of medical research that is conducted at Erasmus Medical Center is one of these objectives. Furthermore, the Editorial Board aims to provide an opportunity for students to learn how to make the transition between assignment-writing and producing publishable academic work. This provides the students with a great learning experience. A learning experience that not only helps the students to get their work published in EJM, but one that also helps the students to publish their work in international medical journals in the future. This learning experience consists of several steps that are quite similar to the submission process of international medical journals. By submitting their article to EJM the students can get acquainted with the submission of articles on a professional level.

First, all submitted manuscripts are reviewed by two reviewers. This team of reviewers always consists of one student reviewer and one member of the faculty staff. It usually takes several revision rounds before the submitted manuscript is considered suitable for publication. After every revision round the authors receive feedback from the reviewers.

Second, each accepted manuscript undergoes English editing which is done by a professional editor. Uniquely, all authors receive extensive individual feedback on their own papers. During the last step the authors are supported in restructuring the texts and making the article suitable for publication. This provides the authors the necessary tools on how to design and organize a scientific article for publication.

The objective of EJM is, therefore, not only to publish papers written by students but also to provide the students with the necessary knowledge and experience that they will need during their entire scientific career. Furthermore, receiving extensive feedback on all the different aspects of the article, gives the students a chance to improve their skills in academic writing. We encourage all students to use this great opportunity and to submit their work to EJM.

Bas Hullegie
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Maartje van der Schaaf
Thomas Thijs

Medical students, Erasmus MC University Medical Center Rotterdam, the Netherlands

A thank you to all

This second issue of the Erasmus Journal of Medicine is the result of the dedicated contributions of many persons.

We thank Ed Hull and Charles Frink for sharing their invaluable editorial experience with us.

The journal was typeset and prepared for printing by Ditems Media, thank you Kim, especially for the way you handled all last minute corrections.

The editorial board of EJM has done a tremendous job, I thoroughly enjoyed the professionalism and enthusiasm of Maarten Frens, Paul van Daele, and our student editors Mostafa Mokhles, Maartje van der Schaaf, Denise van der Linde, Bas Hullegie and Thomas Thijs. I thank our reviewers Konstantinos Vakalopoulos and Renuka Birbal, for making themselves available.
We gratefully acknowledge the continuing support of the dean of Erasmus MC, professor Huib Pols, and of the MFVR, the medical students’ society of Erasmus MC.

The journal would not be here before you without the continuous efforts of Petra Erkens, assistant to the editorial board, who kept us all on track.

But most of all I would like to thank all authors who submitted a paper to the journal. I hope you enjoyed the experience of getting a paper criticized and often improved by the reviewers’ and editors’ comments. Most of you will now see it now published, at last, either on real paper or more likely, as an e-publication, on www.erasmusjournalofmedicine.nl.

Diederik Dippel, co-editor in chief

Erasmus Journal Of Medicine

Image from Myrte Maessen & Beatrice van der Matten, Correlation between prenatal test results and fetal autopsy findings, this journal, page 18

No more biopsies?

Worldwide, hepatitis B and C form a major health problem, leading to liver fibrosis, cirrhosis and hepatocellular carcinoma in many, but not all, patients. Establishing who is at risk for developing end stage liver disease now highly depends on liver biopsy, an invasive procedure prone to complications.

In the article by Stibbe et al, less invasive techniques to establish collagen deposition and thereby fibrosis in the liver were studied. The authors used breath tests, ultrasound and various biochemical markers and compared them with the results of liver biopsies. Especially when combining the results of the various tests they were able to correctly predict the amount of fibrosis in the biopsy samples with an area under the ROC-curve of up to 0.92 depending on the degree of fibrosis.

The results of this study are important. Patients with liver disease often also have coagulation disorders making them even more at risk for post procedure hemorrhage. Applying the results of this study might lead to avoidance of risky procedures. The question arises however whether all the techniques like transient elastography and used are widely available.
In this study only patients with liver disease due to either hepatitis B or C were examined.

The main reason for liver disease in the Western world remains the use of alcohol. The pathogenesis of fibrosis and cirrhosis in toxic hepatitis differs from the process seen in viral hepatitis. Whether results from this study therefore can be applied to other liver disease remains to be elucidated.

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Medicine Based Evidence

In their paper 'Should we record the DNA profiles of the entire Dutch population in a DNA database?', Lisanne Konings and Bernhard van Rossum address a topic where biomedical research deeply influences a societal issue that is not related to medicine itself. Based on purely practical considerations Konings and van Rossum conclude that it makes no sense to aim for a genomic database for the whole Dutch population. To me especially the argument that less than 1% of the crime scenes contains useful DNA, and hence that a huge and costly endeavor would be needed to solve an extremely small number of crimes, was an eye opener.

It is extremely important that medical students and professionals think about such issues. Nowadays medical research is much more than just improving diagnosis and therapy. Health and therefore medicine are increasingly important factors in all aspects of society. Architecture, sustainable economy, education and jurisdiction are just a few of the disciplines that gaze at biomedical research for fundamental answers.

The paper of Konings and van Rossum as well as the paper by Bas Mourik in this same issue were written as part of the 'Medicine Based Evidence' assignment in the Erasmus MC Honours Class. The Honours Class is an initiative to let talented bachelors students investigate exactly such topics, on the points where medicine, science and society meet. They do so by reading papers, have class meetings with leading experts in the field, and conducting interviews with people that are directly involved. The students themselves take a lot of responsibility in making such projects a success.

More information on the Erasmus MC Honours Class can be found at http://www.honoursclassemc.nl/

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Comparison of non-invasive assessment to diagnose liver fibrosis in chronic hepatitis B and C patients

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List of Abbreviations
α2M, alpha 2 Macroglobulin;
ALT, alanine aminotransferase;
ApoA1, apolipoprotein A1;
APRI, AST platelet ratio index;
AST, aspartate aminotransferase;
AUC, area under the curve
BMI, body mass index
CHB, chronic hepatitis B virus;
CHC, chronic hepatitis C virus;
GBT, 13C-galactose breath test;
γGT, gamma-glutamyltransferase;
HA, hyaluronic acid;
IQR, interquartile range;
MBT, 13C-methacetin breath test;
NPV, negative predictive value;
PPV, positive predictive value;
PTT, prothrombin time;
ROC, receiver operating characteristic
TE, transient elastography.

Summary

Objective: **Chronic hepatitis B and C cause liver fibrosis, leading to cirrhosis. Fibrosis assessment is essential to establish prognosis and treatment indication. We compared 7 non-invasive tests, separately and in combination, in chronic hepatitis patients to detect early stages of fibrosis according to the Metavir score in liver biopsy.**
Materials and Methods: **Galactose and methacetin breath tests (GBT, MBT), biomarkers (hyaluronic acid (HA), APRI, FibroTest and Fib-4) and transient elastography (TE) were evaluated in 89 patients. For evaluating breath tests and biomarkers, 31 healthy controls were also included.**
Results: **Serum markers HA, APRI, FibroTest, Fib-4 and elastography significantly distinguished non-cirrhotic (F0123) from cirrhotic (F4) patients ($p<0.001$, $p=0.015$, $p<0.001$, $p=0.005$, $p=0.006$, respectively). GBT, HA, APRI, FibroTest, Fib-4 and TE detected F01 from F234 ($p=0.04$, $p=0.011$, $p=0.009$, $p<0.001$, $p<0.001$, $p<0.001$, respectively). A combination of tests (TE, HA and FibroTest) improved the performance, AUC=0.87 for F234, 0.92 for F34, and 0.90 for F4.**
Conclusion: **HA, APRI, FibroTest, Fib-4 and TE reliably distinguish non-cirrhotic and cirrhotic patients. Except for MBT, all tests discriminated between mild fibrosis and moderate fibrosis. As single tests: FibroTest, Fib-4, and TE were the most accurate for diagnosing early fibrosis. Combining different non-invasive tests increases the accuracy of diagnosis and may reduce the number of liver biopsies.**

Introduction

Chronic viral hepatitis B (CHB) and C (CHC) are public health problems worldwide, leading to liver fibrosis and ultimately to cirrhosis, decompensated liver disease and hepatocellular carcinoma. At present, the golden standard to assess liver fibrosis is a liver biopsy using the Ishak (1) or Metavir (2, 3) fibrosis scoring systems. However, biopsy is prone to sampling error and substantial intra- and inter observer variability, leading to over or under staging of fibrosis (4). This procedure also has significant morbidity, including infections, major bleeding, ascites leakage and postprocedure pain, and can lead to mortality (5); it is contraindicated for patients with a coagulation disorder.

Moreover, liver biopsy is not a perfect golden standard because it sometimes results in false positive and false negative diagnoses (6). This hampers the primary determination of fibrosis and optimal management of therapy. Consequently, there is a need for non-invasive methods to accurately diagnose the presence of liver fibrosis and cirrhosis.

Most previous studies on non-invasive diagnostics, including breath tests, serum biomarkers and transient elastography (TE), reliably determined the presence or absence of cirrhosis, but did not discriminate between the earlier stages of fibrosis very well (7-9).

Breath tests measure various metabolic functions and rely on processing of an administered ^{13}C-labelled substrate (stable isotope), which can be detected in expired air. Expired $^{13}CO_2$ reflects the residual functional liver mass (10).

GBT measures galactose oxidation capacity of the liver in a cytosolic pathway in which galactose kinase is the rate limiting enzyme in the metabolism leading to $^{13}CO_2$ (11).

Methacetin enables quantitative evaluation of the cytochrome P450 IA2 dependent liver function in polymorphic mitochondria. After ingestion of ^{13}C-methacetin, the liver metabolises ^{13}C-methacetin into acetaminophen and $^{13}CO_2$ (12).

With respect to the serological tests, HA is an unbranched high-molecular-weight polysaccharide and a component of the extracellular matrix. The HA levels in serum are elevated in advanced liver diseases, which is caused by increasing amounts of fibroblasts and hepatic stellate cells (13).

The APRI score consists of the AST-to-platelet ratio, two inexpensive laboratory tests which are routinely performed on all patients (9, 14-16).

The FibroTest is an algorithm consisting of age, sex and five serum markers: alpha-2 macroglobulin (α2M), haptoglobin, total bilirubin, gamma-glutamyltransferase (γGT) and apolipoprotein A1 (ApoA1) (17).

The Fib-4 index is a simple and inexpensive algorithm consisting of age and the routine laboratory tests AST, ALT and platelets (18, 19). In addition, the correlation of histology with TE has been evaluated (20).

TE measures liver stiffness in kilopascals (21). With TE, a larger sample size of the liver is examined than in biopsy, which ensures a reduction in sampling error (7, 22). In previous studies, TE has shown good discriminative value for the presence or absence of cirrhosis (F4). Although there is significant overlap between F1, F2 and F3, the accuracy of discrimination is reasonable (23).

The aim of this study was to compare 7 non-invasive diagnostics, the galactose- (GBT) and methacetin breath tests (MBT), four biochemical serum markers (hyaluronic acid (HA), APRI, FibroTest and Fib-4) and TE, separately and in combination, to access liver fibrosis in a single study population. The reference for these non-invasive tests was Metavir staging in the liver biopsy with an adequate length of ≥20 mm.

Methods

Study population

Mono-infected CHB or CHC patients referred for liver biopsy to our out-patient clinic were invited to participate in this cross-sectional study. All included patients were invited to undergo all diagnostic tests. Exclusion criteria were alcohol intake >2 units/day, co-infection with HIV or Hepatitis D, or the presence of hepatocellular carcinoma. Additionally, healthy controls were included for the breath tests and serological tests. The study protocol was approved by the ethics committee of Erasmus MC. All subjects provided written informed consent prior to enrolment.

Liver biopsy

Two experienced hepatologists performed all biopsies. To reduce complications, during this procedure abdominal ultrasound was used to identify liver parenchymal and vascular structures. Biopsies were taken with a 14G true-cut needle and required a length ≥20 mm. After embedding biopsies in paraffin, lengthwise sections were cut and stained with picrosirius red. Two expert hepatopathologists scored all specimens (double read) for different fibrosis categories using Metavir scoring: F0: no fibrosis, F1: portal fibrosis without septa, F2: few septa, F3: numerous septa without cirrhosis, F4: cirrhosis (2). No biopsies were obtained from controls.

Breath tests

Both breath tests required overnight fasting before the test. For GBT, the participant received a dose containing 495 mg/kg unlabelled (^{12}C-) galactose bodyweight (VWR, Prolabo, Amsterdam) and 5 mg/kg ^{13}C-labelled galactose (99% APE, Sigma-Aldrich, Zwijndrecht) in 200 mL water. For MBT, the dose was 2 mg/kg ^{13}C-methacetin (99% APE, Campro, Berlin; N-(4-methoxy-^{13}C-phenyl) acetamide) in 200 mL water. At baseline (T=0 minutes) 4 breath samples were collected, followed by duplicate breath samples at 10, 20, 30, 40, 60, 90, 120, 150 and 180 minutes after drinking the substrate.

To exclude any influence of variations in CO_2 production during the breath tests, participants were at rest, fasting and were not allowed to smoke.

The $^{13}CO_2/^{12}CO_2$ isotope ratio in the breath samples was analysed by isotope ratio mass spectrometry (ABCA Sercon, UK).

The percentage of ^{13}C exhaled was calculated assuming a CO_2 production rate of 9 mmol/hour/kg.

Results were expressed as the cumulative proportion of ^{13}C administered dose recovered over time using area under the curve (AUC) calculations and were categorised by Metavir classification. Elapsed time between GBT and MBT was at least one week to exclude any influence of previous ^{13}C-substrates. In all patients the breath tests were performed within 6 months after liver biopsy irrespective of treatment.

Serum markers

Blood samples were obtained from all patients on the day of biopsy. Blood was taken from healthy controls preceding the breath test. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin and total bilirubin were determined in all participants. γGT, alkaline phosphatase, trombocytes and prothrombin time (PTT) were additionally determined in patients, but not in controls. Serum HA was measured (Corgenix, Broomfield, CO, USA) in all participants (normal range: 0-75 ng/mL).

APRI was calculated in the serum of all patients as follows: AST/(UNL)*100/platelets(10^9/L). FibroTests were performed on all serum samples from patients and controls. This FibroTest was based on sex, age and 5 serum markers: α2M (Dako Diagnostics,

Enschede, the Netherlands), haptoglobin, total bilirubin, γGT (Roche Diagnostics, Maizy, France) and ApoA1 (Beckman Coulter, Wiener Neudorf, Austria). α2M, haptoglobin, total bilirubin, γGT were determined on a Modular P800 system (Roche) and ApoA1 on an Immage 800 system (Beckman). FibroTest results ranged between 0 and 1 (24).

Fib-4 was measured in the serum of all patients as follows: age(yr)* (AST(U/l)/ Platelets(10^9/L)* √ALT(U/l)) (19). For both FibroTest and Fib-4, increasing outcomes corresponded to more severe fibrosis stages.

Transient elastography

TE (FibroScan®, EchoSens, Paris, France) preceded the biopsy in the same session (21, 25). TE measured low-frequency elastic waves (50 Hz) through a medium, and the speed of these waves was positively correlated with stiffness of the liver. A success rate of >60% was considered reliable in 10 validated measurements with IQR <30% of the median (26).

Statistics

The data were analysed using SPSS version 16.0 for Windows. Clinical and laboratory data were shown as mean with 95% confidence interval. Diagnostic results between patients were compared using the non-parametric Wilcoxon-Mann-Whitney U-test or Student's T-test, where two-sided p values ≤0.05 were regarded as significant. Analysis of variance (ANOVA) was used to compare continuous variables. Diagnostic performances of non-invasive tests were expressed by sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and the area under the Receiver Operating Characteristic (ROC) curve. In ROC curves, the true positive rate (sensitivity) is plotted as a function of the false positive rate (100-specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test. To assess the association between non-invasive diagnostics and histology (the golden standard), linear and binary logistic regression analyses were performed.

Results

This study included 89 patients and 31 healthy controls; 48 CHB patients (35 men; mean age 37 years; mean body mass index (BMI) 25.4), 41 CHC patients (27 men; mean age 47 years; mean BMI 25.0), and 31 controls (19 men, mean age 35 years, mean BMI 25.0). All liver biopsies of the patients were scored using the Metavir system by 2 expert pathologists: 9 patients were scored as F0 (no fibrosis), 36 as F1 (mild fibrosis), 14 as F2 (moderate fibrosis), 14 as F3 (moderate to severe fibrosis), and 16 as F4 (cirrhosis). All cirrhotic patients were classified as Child Pugh A. Additional demographic, viral and biochemical characteristics of the study population are shown in Table 1. Because of logistic or medical reasons, not all patients underwent all non-invasive tests.

Breath Tests

None of the participants sustained any adverse reactions, such as diarrhoea or abdominal pain, as a result of the galactose or methacetin intake. The numbers of participants per test are shown in Table 2. For GBT, the mean of cumulative recoveries in controls was higher than in patients (p<0.001). GBT distinguished F01 from F234 (p=0.04), but did not reliably distinguish F0123 from F4 (p=0.12). Using linear regression, cumulative recovery (AUC) of GBT was on average 0.29 lower in men than in women (p=0.002) and was inversely related with age (0.27; p<0.001). MBT did not significantly distinguish F01 from F234 or F0123 from F4 (Table 2). Using linear regression, MBT was positively correlated with BMI (0.24; p=0.011), data not shown.

Serum Markers

Because no biopsies were taken from the controls, AST, ALT, albumin, and total bilirubin were measured to confirm them as healthy. HA was performed on all participants (Table 2, Fig. 1A).

Table 1 - Demographic, viral and biochemical characteristics of CHB and CHC patients and controls

		Metavir (n=89)				
	Controls (n=31)	F0 (n=9)	F1 (n=36)	F2 (n=14)	F3 (n=14)	F4 (n=16)
*Demography***						
Male	19 (61%)	5 (56%)	23 (64%)	12 (86%)	10 (71%)	12 (75%)
*Age (yr)**	35 ± 11	36 ± 11	36 ± 11	44 ± 14	48 ± 11	48 ± 9
*Height (cm)**	175 ± 10	171 ± 9	172 ± 10	174 ± 7	170 ± 12	176 ± 13
*Weight (kg)**	77 ± 14	70 ± 12	74 ± 15	73 ± 12	78 ± 10	80 ± 13
*BMI**	25 ± 4	24 ± 3	25 ± 4	24 ± 4	27 ± 4	26 ± 4
*Etiology***						
CHB	-	6 (67%)	20 (56%)	10 (71%)	7 (50%)	5 (31%)
CHC	-	3 (33%)	16 (44%)	4 (29%)	7 (50%)	11 (69%)
Biochemistry†*						
AST (U/l)	30 ± 10	35 ± 10	50 ± 42	66 ± 45	52 ± 22	68 ± 46
ALT (U/l)	29 ± 22	53 ± 25	84 ± 99	111 ± 111	78 ± 48	55 ± 40
Albumin (g/l)	46 ± 2	46 ± 2	46 ± 3	45 ± 3	44 ± 2	42 ± 5
Tot Bilirubin (µmol/l)	12 ± 6	14 ± 9	12 ± 9	13 ± 6	13 ± 7	16 ± 10
γGT (U/l)	-	31 ± 11	54 ± 53	48 ± 38	82 ± 91	125 ± 153
Alk. Phosphatase (U/l)	-	69 ± 16	69 ± 16	74 ± 18	69 ± 17	87 ± 40
*Trombocytes (*10E9/l)*	-	228 ± 57	216 ± 59	230 ± 69	188 ± 44	140 ± 85
PTT (sec)	-	12 ± 1	12 ± 1	12 ± 1	12 ± 1	14 ± 2

* Mean ± standard deviation; ** Numbers (percentage of subgroup)

† Normal reference ranges: AST: <37 U/l for men, <31 U/l for women; ALT: <41 U/l for men, <31 U/l for women; Albumin: 35-50 g/l; Total Bilirubin: <17 µmol/l; γ-GT: <50 U/l; Alk.Phosphatase: <120U/l; Trombocytes: 150-400*109/l; PTT: 10.9–13.3 sec.

Table 2 - Outcome of the non-invasive diagnostics in controls and patients, according to Metavir stages

		Metavir (n=89)					*p-value***	*p-value***
Total	Controls (n=31)	F0 (n=9)	F1 (n=36)	F2 (n=14)	F3 (n=14)	F4 (n=16)	≥F2	≥F4
GBT*	20.4±3.4	17.8±3.9	16.5±4.2	15.3±3.1	15.2±3.7	14.3±5.3	0.04	0.118
	(n=31)	(n=8)	(n=34)	(n=14)	(n=11)	(n=15)		
MBT*	68.2±15.5	74.3±17.3	70.8±17.0	68.1±15.3	63.6±12.	63.0±21.7	0.083	0.189
	(n=30)	(n=9)	(n=31)	(n=14)	(n=11)	(n=15)		
HA*	23.8±27.7	27.4±18.5	41.1±61.7	31.8±30.2	65.8±75.7	182.4±190.	0.011	<0.001
APRI*	-	0.4±0.2	0.7±0.6	0.8±0.5	0.8±0.3	2.0±1.8	0.009	0.015
FibroTest*	0.11±0.09	0.21±0.17	0.21±0.17	0.33±0.21	0.45±0.20	0.55±0.26	<0.001	<0.001
Fib-4		0.85±0.41	1.03±0.53	1.4±0.77	1.60±0.46	5.01±4.70	<0.001	0.005
TE*		6.4±1.0	7.6±4.6	10.2±5.6	14.1±7.0	22.9±16.2	<0.001	0.006
		(n=8)	(n=34)	(n=11)	(n=13)	(n=15)		

* Mean ± standard deviation. ** Control group excluded from statistical analysis.

TAble 3 - AUC and 95% CI for GBT, MBT, HA, APRI, FibroTest, Fib-4 and TE, according to Metavir stages

	≥F2(95% CI)	≥F3(95% CI)	≥F4(95% CI)
GBT	**0.62**(0.50-0.75)	**0.60**(0.47-0.74)	**0.61**(0.44-0.79)
MBT	**0.62**(0.49-0.74)	**0.62**(0.49-0.75)	**0.58**(0.42-0.74)
HA	**0.72**(0.61-0.82)	**0.81**(0.72-0.91)	**0.86**(0.75-0.96)
APRI	**0.69**(0.58-0.80)	**0.69**(0.57-0.81)	**0.72**(0.55-0.89)
FibroTest	**0.80**(0.71-0.89)	**0.83**(0.74-0.92)	**0.81**(0.70-0.92)
Fib-4	**0.80**(0.71-0.89)	**0.82**(0.74-0.91)	**0.84**(0.72-0.97)
TE	**0.83**(0.74-0.93)	**0.87**(0.79-0.96)	**0.89**(0.82-0.96)
HA+ FibroTest	**0.81**(0.71-0.90)	**0.85**(0.76-0.93)	**0.86**(0.76-0.96)
HA+ Fib-4	**0.80**(0.71-0.90)	**0.83**(0.75-0.92)	**0.86**(0.74-0.98)
HA+ TE	**0.85**(0.76-0.94)	**0.91**(0.85-0.98)	**0.91**(0.84-0.97)
FibroTest+ Fib-4	**0.83**(0.74-0.91)	**0.86**(0.78-0.94)	**0.87**(0.76-0.97)
FibroTest+ TE	**0.86**(0.78-0.95)	**0.90**(0.84-0.97)	**0.87**(0.78-0.95)
Fib-4+ TE	**0.88**(0.80-0.96)	**0.90**(0.83-0.96)	**0.90**(0.81-0.99)
HA+ FibroTest+ TE	**0.87**(0.78-0.95)	**0.92**(0.86-0.98)	**0.90**(0.83-0.98)
HA+ FibroTest+ Fib-4	**0.83**(0.75-0.92)	**0.86**(0.78-0.94)	**0.87**(0.77-0.98)
HA+ FibroTest+ Fib4+ TE	**0.87**(0.79-0.96)	**0.91**(0.85-0.97)	**0.91**(0.82-0.99)

HA distinguished F0123 from F4 (p<0.001) with a corresponding AUC mean (95% CI) of 0.86 (0.75-0.96) (Fig. 2C) and F01 from F234 (p=0.011) with AUC of 0.72 (95% CI: 0.61-0.82) (Fig 2A, Table 3). Despite the significance, almost all measurements in the range F0-F3 were within normal values (≤75 ng/mL). APRI scores were calculated only for patients (Table 2). APRI significantly distinguished F0123 from F4 (p=0.015), F012 from F34 (p=0.013) and F01 from F234 (p=0.009), despite some overlap between the various stages of fibrosis.

In the controls, the FibroTest results did not differ from F0 (p=0.127), as expected (Table 2, Fig. 1B). FibroTest discriminated very well between F0123 and F4 (p<0.001). Also, F01 was significantly different from F234 (p<0.001) with an AUC of 0.80 (0.71-0.89) (Table 3). After exclusion of severe fibrosis and cirrhosis, patients with F01 differed from F2 (p=0.024). Even F1 by itself was different from F2 (p=0.032). The optimal cut-off values for several fibrosis stages with corresponding sensitivity, specificity, PPV and NPV are show in Table 4A. Using 0.75 as cut-off value in detecting cirrhosis, sensitivity and NPV were 1 for both CHB and CHC. F34 was detected with a high sensitivity of 97% and 91% in CHB and CHC, respectively, and F234 was detected with a sensitivity of 85% and 74%. Using linear regression, the FibroTest correlated positively with histology (p<0.001). Fib-4 scores were calculated for all patients (Table 2, Fig 1C). The values ranged from 0.28 to 15.25.

Table 4 - Diagnostic values of non- invasive tests with defined optimal cut-off.

A. FibroTest (CHB n=48; CHC n=40)

	≥F2		≥F3		≥F4	
	HBV	HCV	HBV	HCV	HBV	HCV
Cut-off*	0.31	0.31	0.58	0.58	0.75	0.75
Sens.	0.85	0.74	0.97	0.91	1.00	1.00
Spec.	0.68	0.76	0.25	0.41	0.08	0.24
PPV	0.76	0.74	0.80	0.68	0.77	0.64
NPV	0.79	0.76	0.75	0.78	1.00	1.00

B. Fib-4 in detecting fibrosis (≥F3) (CHB n=48; CHC n=41)

	HBV		HCV	
Cut-off**	<1.45	>3.25	<1.45	>3.25
Sens.	66.7	25	72.2	27.8
Spec.	80.6	97.2	69.6	100
PPV	53.3	75	65	100
NPV	87.9	79.5	76.2	63.9

C. Transient elastography (CHB n=45; CHC n=36)

	≥F2		≥F3		≥F4	
	HBV	HCV	HBV	HCV	HBV	HCV
Cut-off***	7	5	10	10	14	14
Sens.	0.73	0.06	0.85	0.84	0.80	0.88
Spec.	0.84	1.00	0.73	0.76	0.75	0.73
PPV	0.86	1.00	0.91	0.80	0.97	0.88
NPV	0.70	0.57	0.62	0.81	0.27	0.73
LR +	4.63	0.06	3.13	3.58	3.22	3.23
LR -	0.32	0.94	0.20	0.21	0.26	0.16

Values defined by Poynard et al*, Sterling et al**, Verveer*** (personal communication).
Sens: Sensitivity; Spec: Specificity; PPV: positive predictive value, NPV: negative predictive value; LR +: positive likelihood ratio, LR -: negative likelihood ratio.

Fib-4 differentiated between several fibrosis stages: F0123 from F4 (p=0.005), F012 from F34 (p=0.013) and F01 from F234 (p<0.001). Also, no fibrosis and mild fibrosis (F01) was distinguished with statistical significance from moderate fibrosis (F2) (p=0.03). Fib-4 correlated positively with FibroTest (63.7%), TE (58%) and histology (60%). No patients with F34 had a Fib-4 value <0.8; therefore the NPV was 100%.

Cut-off values of 1.45 and 3.25 for predicting severe fibrosis had previously been determined by Sterling (19). Using 3.25 as cut-off in our data set showed a specificity and NPV in CHC of both 100% (Table 4B). Using 1.45 in CHC, the sensitivity was 72.2% and PPV was 65%. 63.4% of all Fib-4 results in CHC were not ranged between both cut-offs, so patients could be prevented from undergoing biopsy with 19% misclassification rate.

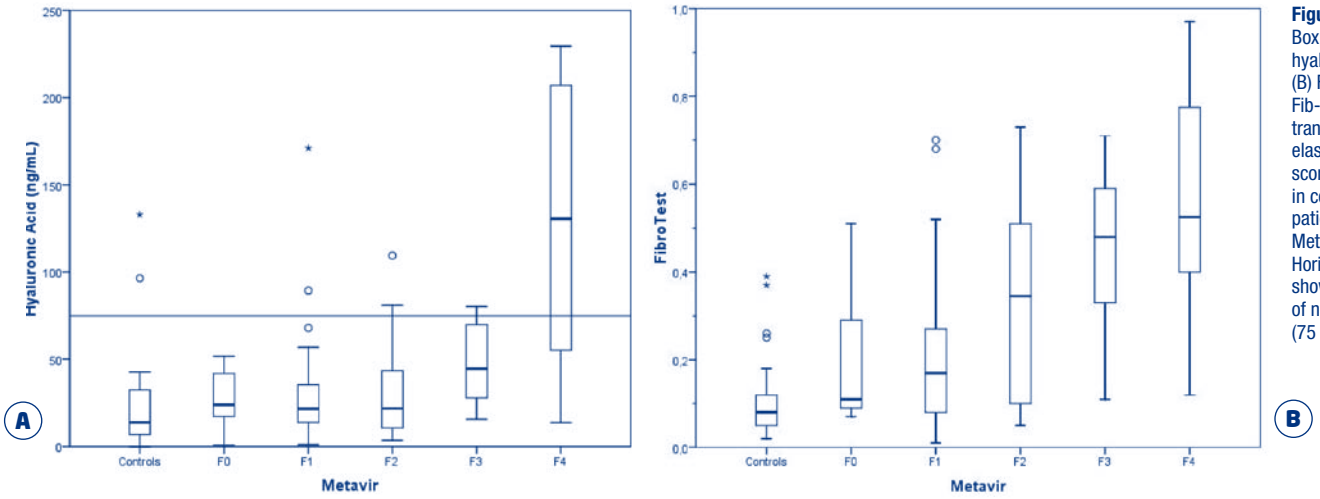
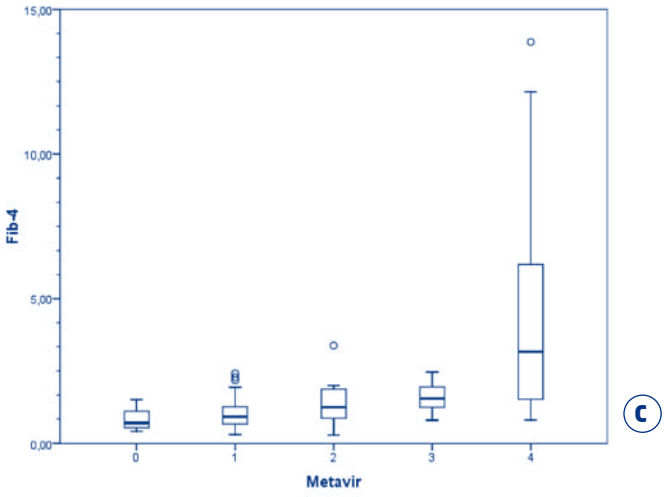


Figure 1
Boxplots of (A) hyaluronic acid, (B) FibroTest, (C) Fib-4 and (D) transient elastography scores performed in controls and patients by Metavir stage. (A) Horizontal line shows upper limit of normal range (75 ng/mL).

In CHB, the cut-off at 3.25 showed specificity of 97.5% and NPV of 79.5%, whereas the cut-off at 1.45 showed specificity of 80.6% and NPV of 87.9%. Using these cut-off values, only 14% of the samples were misclassified compared with liver biopsy, and 77% of the non-invasive tests correspond to biopsies.

Transient Elastography

Elastography was performed in 81 patients, where 8 patients were excluded from TE due to failure of adequate measurements. TE distinguished between F0123 and F4 ($p<0.006$), between F012 and F34 ($p<0.001$), as well as between F01 and F234 ($p<0.001$) (Table 2, Fig. 1D). Even after exclusion of F4, patients with F23 showed increased elasticity compared to F01 ($p=0.001$). The corresponding AUC mean (95% CI) with threshold F34 was 0.87 (0.79-0.96) and with threshold F4 it was 0.89 (0.82-0.96) (Fig. 2, Table 3). TE was confounded by age ($p=0.010$). Cut-off values for several fibrosis stages, subdivided by aetiology, were defined by Verveer (unpublished data) (Table 4C).



Combination of non-invasive methods

Using ordinal logistic regression, the FibroTest and Fib-4 by themselves both showed an AUC of 0.80 for F01 vs. F234. TE showed an AUC of 0.83, which was not significantly different than the AUC of FibroTest or Fib-4 ($p=0.20$, $p=0.34$ respectively). Several combinations of non-invasive tests were tested (Table 3). Combinations with breath tests or APRI did not improve the AUC (data not shown). Fib-4 and TE combination increased the AUC to 0.88 (0.80-0.96) for F01 vs. F234. In contrast, adding GBT, MBT, HA, APRI or FibroTest to Fib-4 and TE did not improve the AUC for ≥F2, or improved it only slightly.

HA, FibroTest, Fib-4 and TE were good markers for detecting severe fibrosis ≥F3. These tests, expressed as AUC, were significantly higher than both GBT and MBT (HA: $p=0.009$ and $p=0.0003$; FibroTest: $p=0.039$ and $p=0.011$; Fib-4: $p=0.018$ and $p=0.0008$; TE: $p=0.0005$ and $p=<0.0001$, respectively). The best combination, consisting of HA, FibroTest and TE, increased the AUC to 0.92 for ≥F3. The percentage of patients classified correctly with concordant test results was 58%. The number of misclassification was only 4%. If only two out of three tests had to be conclusive, the number of correctly classified patients increased to 80%, with a sensitivity of 52%, specificity of 96%, PPV of 88% and NPV of 80%.

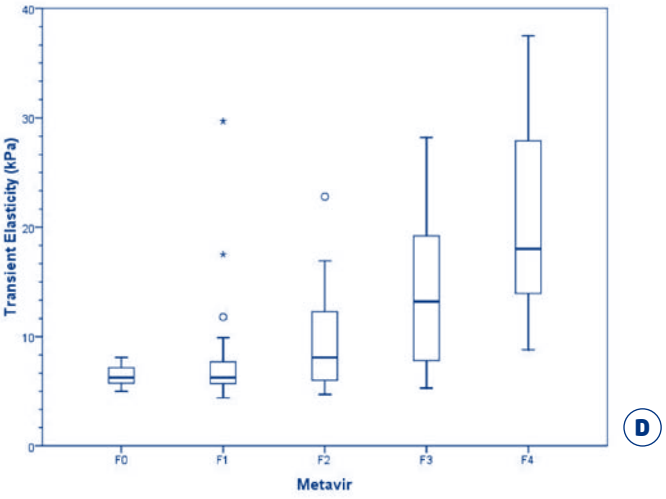
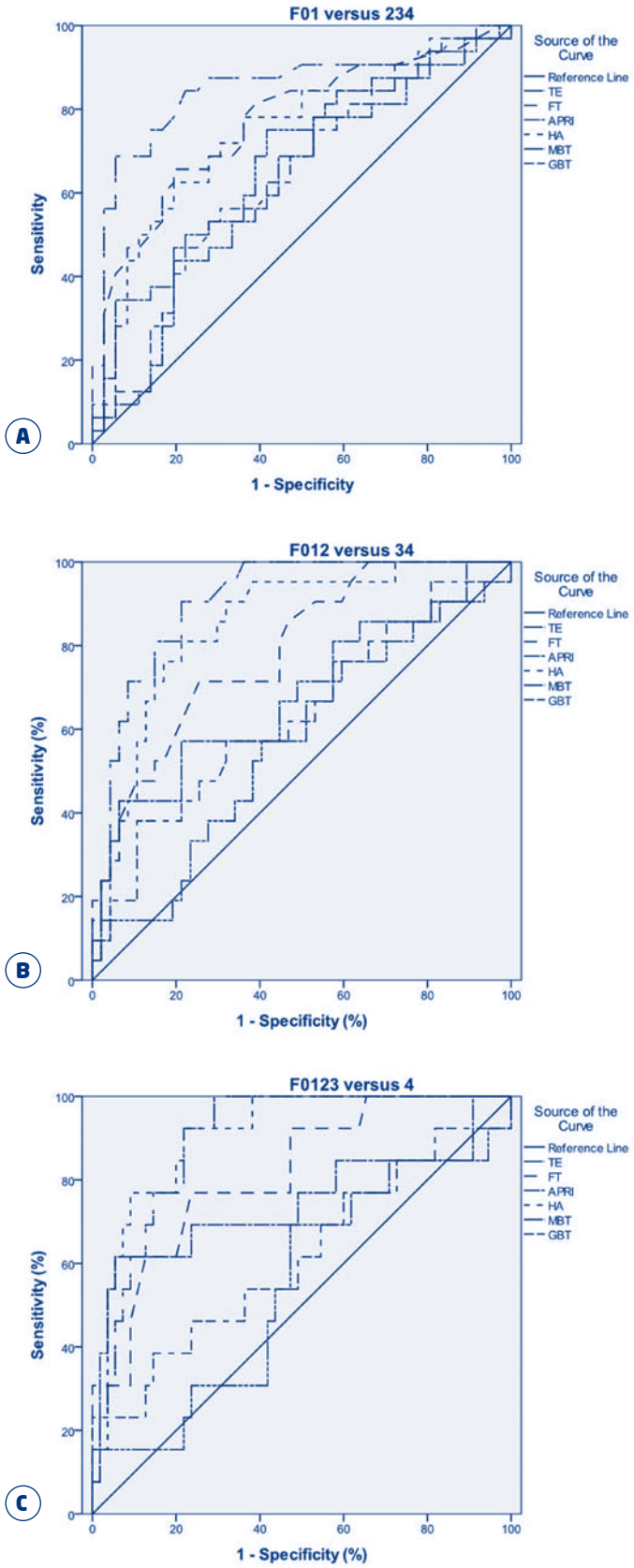


Figure 2
Receiver operator characteristic (ROC) curves from all non-invasive tests with (A) threshold F01 versus F234, (B) F012 versus F34 and (C) F0123 versus F4. Patients with missing data were not included. Diagonal segments are produced by ties.



Cirrhosis detection ≥F4 was very accurate using TE (AUC of 0.89). TE combined with HA increased AUC to 0.91. Using this combination, 73% of all patients were classified correctly with a specificity of 75% and a sensitivity of 60%. Misclassification with concordant test results was only 5%.

Discussion

In this study we have shown that all serum markers and TE determine significant fibrosis and cirrhosis with good discriminative value. Most patients participate both galactose- and methacetin breath tests.

In contrast to previous studies (8, 10-12), the results from both our breath tests were disappointing. A specific problem with GBT is the influence of portal blood flow, which is resolved by saturating the liver with high doses of unlabeled galactose. GBT distinguishes reliably between F01 and F234, but not between other fibrosis stages, probably due to the metabolic function overcapacity of the liver. In our study, MBT did not reliably distinguish between any fibrosis stages and was positively correlated with BMI. Significant differences in GBT measurements after 60 min and in MBT after 30 min have been described (11), but analysing these earlier time points in our data set did not result in significance.

HA is a very sensitive marker to exclude cirrhosis, if 75 ng/mL is measured as the upper limit of normal. Several studies have been performed with HA using other cut-off values to exclude cirrhosis. However, in our data set these previously described cut-offs were less sensitive in excluding cirrhosis (27). Fib-4 has already been tested in CHB and CHC co-infected and mono-infected patients separately (28). In our study, we validated Fib-4 in an external data set for both CHB and CHC. Beside AST and platelet count, Fib-4 also takes ALT and age into account, and is therefore much more accurate in diagnosing fibrosis than the APRI score. However, all serum markers and TE are influenced by the presence of inflammation due to additional diseases like rheumatoid arthritis. These influences are observed more frequently in CHC patients, due to the more frequent extra-hepatic inflammatory implications in CHC patients compared to CHB patients.

Despite the small sample sizes, we were able to distinguish between CHB and CHC results. For both the FibroTest and TE, it is important to choose a cut-off value with a high NPV, which results in high specificity for measuring early fibrosis stages. However, in detecting cirrhosis, a high PPV without false negative results is preferable, due to the risk of hepatocellular carcinoma. Of all tested non-invasive diagnostics, TE performed the best as single test.

TE combined with FibroTest and HA increased the accuracy (AUC of 0.92) of predicting severe fibrosis and narrowed the confidence intervals.

In predicting F01 vs. F234, Fib-4 combined with TE performed well, with an AUC of 0.88. However, in clinical practice the same combination of TE, FibroTest and HA can also be used to distinguish F01 from F234 (AUC of 0.87). The AUC of the combination TE, Fibro Test and HA is not statistically different from Fib-4 combined with TE. Unfortunately, elastography is not available in all hospitals. Therefore, the combination of FibroTest and Fib-4 is a good alternative, with an AUC of 0.83. To distinguish between F012 and F34, the best combination was HA, FibroTest and TE (AUC of 0.92). The combination of HA and TE (AUC of 0.91) was the best option to determine cirrhosis.

In addition, conventional ultrasound, used during biopsy sampling, can be used on its own to identify cirrhosis. These ultrasonographic measurements identified cirrhosis in HA>75ng/mL with PPV of 100% and NPV of 97.5%. Ultrasound reports have mentioned cirrhosis in CHC patients (HA>75ng/mL and FibroTest>0.75) with PPV of 100%, although this subgroup consisted of only four

patients. Therefore, as an additional non-invasive diagnostic test to these non-invasive markers, we recommend further research into ultrasonographic measurements in a larger CHC cohort.

Recent studies have reported using APRI and FibroTest (29, 30) as non-invasive diagnostics. These studies support our data concerning the percentage of replacing biopsies in a larger patient cohort.

In conclusion, the AUCs of the non-invasive tests are good, but are not as effective for measuring the early stages of fibrosis (F0, F1 and F2). However, our study has generated promising hypotheses for further research in this field.

Although liver biopsy remains necessary to assess fibrosis in new patients, we recommend combining the first liver biopsy with non-invasive tests. Combinations of TE, HA and FibroTest are accurate enough by themselves to detect moderate and severe fibrosis. Especially when these non-invasive tests correspond with initial histology, non-invasive diagnostic tests can help in future follow-up of disease management, thereby reducing the number of biopsies.

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Original Contribution

Correlation between prenatal test results and foetal autopsy findings

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Research project performed at CGC Genetics in Porto, Portugal, February-July 2010 (21 weeks)
Research fields: Medical genetics, prenatal screening and diagnosis, foetal pathology

Abstract

Objective: **To compare the results of prenatal screening and diagnostics to findings of the pathological examination after foetal demise. Our aim was to evaluate the additional value of the foetal pathological examination for final diagnosis and family counselling.**
Study design: **Retrospective cohort study of patient data obtained from all foetal pathological examinations performed at CGC Genetics in Porto during 2009.**
Materials and methods: **A database containing information on all 454 cases of foetal pathological examinations performed in 2009 was compiled. Of these, 161 cases met the inclusion criteria for analysis: information on foetal malformations found during prenatal ultrasound examination or with prenatal diagnostic techniques was available. To compare the autopsy findings to the results of prenatal screening/diagnostics, the cases were assigned to one of four subgroups according to their degree of concordance.**
Results: **Autopsy findings were concordant with prenatal results in all cases but one. Autopsy provided relevant additional findings in 29% of the cases and minor additional information in 11%. The rate of complete concordance was 59%. Apart from chromosomal abnormalities and single-gene defects, the rate of concordance was highest in cases of nervous system anomalies. In cases with cardiac, renal or digestive malformations, autopsy provided relevant additional information in more than half of the cases.**
Conclusion: **This study reinforces the value of foetal autopsy in medical genetic counselling after foetal demise by providing a diagnosis and by determining recurrence risk for future pregnancies.**

Introduction

Major congenital malformations in foetuses make an important contribution to perinatal mortality rates. The prevalence of major congenital malformations in Europe is 23.8 per 1000 births. For live births this prevalence is 19.9 per 1000 births. The main causes of congenital malformations are structural heart disease (0.23/1000 births), chromosomal abnormalities (0.21/1000 births) and central nervous system defects (0.19/1000 births)(1).

During the last decade, prenatal screening methods and protocols have developed substantially. In most European countries, second trimester ultrasound screening is accessible to all pregnant women (2). Even earlier in the pregnancy, a calculation of the risk of Down's syndrome can be made by combined ultrasonographic and biochemical screening methods.

When a congenital malformation or chromosomal abnormality is detected by prenatal ultrasound or diagnostic methods, many couples consider termination of the pregnancy. In order for couples to make a well-considered decision, they should be offered medical genetic counselling. During this counselling, a medical geneticist aims to provide information about the clinical features, causes and treatment of the malformation, explain the heredity of a genetic disease or recurrence risk of a malformation, and help the couple in the emotional process of decision making (3).

However, in some of the cases there is no definite prenatal diagnosis, for example when a cystic hygroma is seen on ultrasound, or when a cardiac malformation is suspected. In these cases, the consequences of the detected malformation for the child and its recurrence risk are not exactly known. Therefore, especially in these, but in general in all cases, pathological examination of the foetus (autopsy) after termination of pregnancy can provide essential information: a definitive diagnosis and its recurrence risk for future pregnancies (4,5).

Although identification of malformations by means of ultrasound screening and maternal serum markers testing has limitations, the rates of foetal autopsy are declining in Europe. Possible explanations for this decline could be the improvement in prenatal diagnosis techniques and adverse publicity on inappropriately conducted autopsies (4,6).

In our study we first processed the information from all foetal autopsies performed at CGC Genetics in 2009 into a database and selected cases for analysis. Subsequently, we compared the results of prenatal screening and diagnostics to the findings of the pathological examination of the foetus after termination of pregnancy, spontaneous pregnancy loss, intrauterine foetal death, stillbirth or early neonatal death. Our aim was to evaluate the additional value of the foetal pathological exam for final diagnosis and family counselling.

We addressed the following research questions: a) Does foetal autopsy provide additional information, b) is this information relevant in terms of changing the recurrence risk and therefore, is autopsy needed for proper family counseling? c) Should autopsy be performed in all cases of foetal demise?

Materials and methods

We retrieved all foetal pathology cases of 2009 from the archive at CGC Genetics and compiled a database using the Microsoft Access database programme. This resulted in information on 454 cases.

Inclusion and exclusion criteria

Cases of foetal autopsy after termination of pregnancy due to medical reason (TOP), spontaneous pregnancy loss, intrauterine foetal death (IUFD), stillbirth or early neonatal death, for which records on foetal malformations found by prenatal screening or diagnostic tests were available, were included.
Cases for which no clinical information about prenatal results was available were excluded. Cases with only non-foetal abnormalities detected by ultrasound, such as placental abnormalities, amniotic liquid aberrations and suspected molar degeneration, were excluded. When only an ultrasound finding was described that could be a physiological variation, e.g. increased nuchal translucency, the case was also excluded.

Analysis

Analysis was performed by establishing groups of cases according to the organ system of the foetal malformation (organ system subgroups were derived from the EUROCAT-classification (1)). Each case was studied separately, describing what information was known prenatally and after autopsy. We determined whether the information that could be given during medical genetic counselling of the parents was different after autopsy. This information comprised the definitive diagnosis, its clinical features and lethality, its recurrence risk for future pregnancies and whether the parents and other family members should undergo clinical examinations or genetic tests to exclude or prove a specific origin of the foetal malformation.

The information about clinical features, origins and recurrence risks was retrieved from the books *'Oxford desk reference, clinical genetics'* by Firth & Hurst (7) and (1)*Practical genetic counselling' by Harper*(8), from medical genetic information databases GeneReviews (9) and OMIM (Online Mendelian Inheritance in Man) (10) and from medical studies found in the PubMed international database of literature.

According to the degree of concordance between prenatal and autopsy results, the cases were assigned to one of the four following subgroups:

1. Complete concordance
2. Partial concordance, additional information from the autopsy was not clinically relevant
3. Partial concordance, with clinically relevant additional findings from the autopsy (change in recurrence risk or clinical consequences for the couple; e.g. genetic tests, cardiac ultrasounds, blood perfusion tests)
4. Discordance

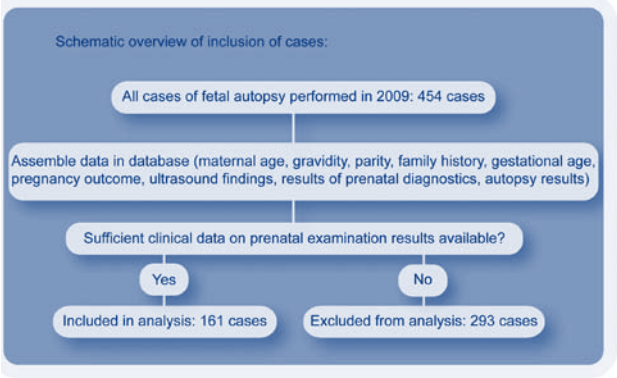
All cases were reviewed by both researchers. If we had any doubt about the assignment of a case into one of the subgroups, then the case was discussed with the pathologist and/or with the clinical geneticist to determine the effect of the additional findings on the recurrence risk, and thus, the consequences for genetic counselling. Statistical analysis was not performed; the results will be displayed descriptively.

Analysis of subgroups

To display the results, percentages of cases assigned to the four subgroups are shown, followed by a subdivision into organ systems. The organ system subgroups with the largest numbers of cases and some particularly interesting cases will be discussed in more detail.

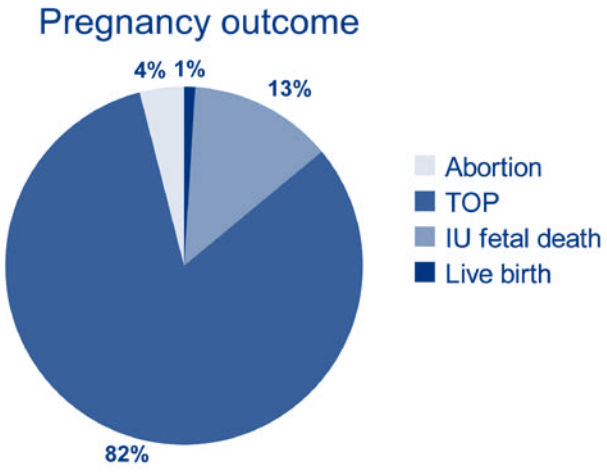
Results

Figure 1 - Overview of inclusion of cases



In 293 cases, no information or insufficient information was provided on the results of prenatal examinations. Most of these concerned spontaneous abortions at short gestational age. Probably, no prenatal screening had been performed as yet in these cases. The remaining 161 cases were included for analysis (Fig. 1). In 111 cases, a foetal malformation was detected by ultrasonographic examination; in 75 cases an abnormality was found by prenatal diagnostic techniques.

Figure 2 - Distribution of the type of pregnancy outcome in the 161 included cases (TOP: termination of pregnancy for medical reason, IU: intrauterine)



Prenatal findings

Ultrasonographic examination

The cases were categorized into organ system subgroups according to the anomalies found during ultrasound examination. When malformations in more than one organ system were identified, an additional second and third organ system could be classified. In Table 1, only the organ system of the primary malformation is shown.

Table 1 - Organ system of the primary malformation found by prenatal ultrasound

Organ system (primary malformation)	n	%
Nervous system	32	28.8
Heart	18	16.2
Respiratory system	1	0.9
Digestive system	4	3.6
Abdominal wall	8	7.2
Urinary system	9	8.1
Limb	5	4.5
Musculoskeletal	1	0.9
Other anomalies	33	29.7
Total	111	100

The subgroup 'other anomalies' contains abnormalities that did not fit into one specific organ system, e.g. lymphatic anomalies (cystic hygroma) and intrauterine growth restriction.

Prenatal diagnosis

In 75 cases, a prenatal diagnosis was made by karyotyping, FISH (Fluorescent In Situ Hybridization)- screening for aneuploidies or molecular diagnosis of a specific disease or mutation. In 71 cases (94.7%), a chromosomal abnormality was found, e.g. a trisomy, triploidy or duplication. In the remaining 4 cases, a single-gene defect was identified, e.g. a homozygous deletion in the SMN1-gene (Survival Motor Neuron 1-gene), causing muscular atrophy.

Autopsy findings

The cases were categorized into organ system subgroups according to the anomalies found during autopsy. When malformations in more than one organ system were identified, an additional second and third organ system could be classified. In Table 2, only the organ system of the primary malformation is shown. The diagram (Fig. 3) also shows the organ systems of the additional malformations.

Figure 3 - Diagram showing the distribution of the organ systems of the primary and additional malformations found at autopsy

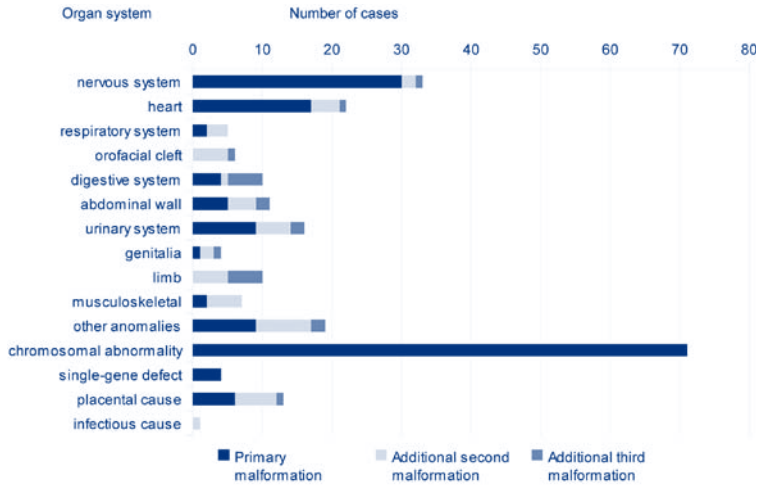


Table 2 - Organ system of the primary malformation found by prenatal ultrasound

Organ system (primary malformation)	n	%
Nervous system	30	18.6
Heart	17	10.6
Respiratory system	2	1.2
Digestive system	4	2.5
Abdominal wall	5	3.1
Urinary system	9	5.6
Genitalia	1	0.6
Musculoskeletal	2	1.2
Other anomalies	9	5.6
Chromosomal abnormality	71	44.1
Single-gene defect	4	2.5
Placental cause	6	3.7
Unclassified	1	0.6
Total	161	100

In the case that could not be classified, the autopsy was severely limited due to a papyraceous foetus.

Subgroups of concordance

According to the degree of concordance between prenatal and autopsy results, the cases were assigned to one of the four subgroups of concordance (as described in Materials and methods).

Table 3 - Assignment of the cases into subgroups of concordance, per organ system of the primary malformation found at autopsy

Organ system (Primary malformation)	Subgroup of concordance (n) 1	2	3	4	Total n (%)
Nervous system	16	5	9	-	30 (18.6)
Heart	5	2	9	1	17 (10.6)
Respiratory system	1	-	1	-	2 (1.2)
Digestive system	-	-	4	-	4 (2.5)
Abdominal wall	1	3	1	-	5 (3.1)
Urinary system	2	2	5	-	9 (5.6)
Genitalia	-	1	-	-	1 (0.6)
Musculoskeletal	-	-	2	-	2 (1.2)
Other anomalies	1	5	3	-	9 (5.6)
Chromosomal abnormality	65	-	6	-	71 (44.1)
Single-gene defect	4	-	-	-	4 (2.5)
Placental cause	-	-	6	-	6 (3.7)
Unclassified	-	-	-	-	1 (0.6)
Total number of cases	95	18	46	1	161 (100)
Percentage	59.0%	11.2%	28.6%	0.6%	100%

Discordance

In one case, prenatal diagnosis and autopsy results disagreed (concordance subgroup 4). At amniocentesis, a trisomy 15 was detected, but cytogenetic analysis after termination of the pregnancy revealed a normal 46,XX karyotype. This pregnancy was initially a twin pregnancy, with early loss of one foetus. Possibly, the material retrieved at amniocentesis came from the vanishing twin. During autopsy of the second foetus, tetralogy of Fallot, pyloric stenosis and pulmonary immaturity were found. No tissue of the first foetus was found during autopsy.

Description of largest organ system subgroups

Chromosomal abnormality

A chromosomal abnormality was found in 71 cases. Trisomy 21 was the most common result. In 41 cases, abnormalities were found at prenatal ultrasound. The most commonly detected anomalies were: increased nuchal translucency, cystic hygroma and fetal hydrops/anasarca. In the remaining 30 cases, it was unclear to us whether an ultrasound was performed.

The diagnosis was prenatally detected in 66 cases. The type of prenatal diagnostic technique was either amniocentesis (38 cases) or chorionic villous sampling (6 cases). In 22 cases, the PND-type was unknown.

During autopsy, foetal anomalies related to the karyotype were identified in all cases. In 65 cases (91.6%), autopsy did not provide additional information (concordance subgroup 1). In 6 cases (8.5%), autopsy provided relevant additional information: 5 diagnoses of chromosomal abnormality and 1 Parvovirus B19 infection as the cause of intrauterine foetal death.

Nervous system

In 30 Cases, nervous system anomalies were identified during autopsy. The most common findings were acrania/anencephaly and holoprosencephaly.

Prenatal ultrasound was performed in all cases. In only one case, no nervous system anomaly was seen on the ultrasound. In this case a cystic hygroma and anasarca were found. In 2 cases an amniocentesis excluded a chromosomal abnormality.

Additional anomalies were found during autopsy in several cases: renal anomalies, abdominal wall defects, cardiac malformations, limb anomalies and genital anomalies. In 5 cases, the additional information was not clinically relevant (concordance subgroup 2). In 9 cases, the autopsy revealed additional information that changed the recurrence risk (concordance subgroup 3): specification of the diagnosis in 3 cases, identification of aqueduct stenosis as a cause of hydrocephalus/ventriculomegaly in 4 cases and discovery of relevant additional malformation in 2 holoprosencephaly cases.

Remarkably, all 9 cases of acrania/anencephaly were detected by prenatal ultrasound.

Heart

A congenital heart malformation was found during autopsy in 17 cases. The most common diagnoses were hypoplastic left heart syndrome and ventricular septal defect.

In one case the mother was affected: she had mitral valve prolapse. The foetus was found to have mitral valve dysplasia and agenesis of the right hand. Maternal diabetes was present in two other cases.

Prenatal ultrasound was performed in 16 cases. In 13 cases, a cardiac malformation was detected. Additional anomalies identified were: facial clefts, digestive system malformations, musculoskeletal and limb anomalies and 'other' anomalies (e.g. foetal hydrops). In the other 3 cases, a limb malformation, a cystic hygroma with anasarca and an echogenic intracardial focus were found.

Prenatal diagnostic techniques were performed in 5 cases, of which one was abnormal: 47,XX,+15 (this was the discordant case).

In 5 cases, complete concordance between prenatal and autopsy results was found. Of these, 4 were cases of hypoplastic left heart syndrome, the 5th case involved polymalformation syndrome. In 9 cases, additional anomalies were found that changed the recurrence risk (concordance subgroup 3), most often involving definition of the cardiac diagnosis.

Discussion

In our study, data from all 454 cases of foetal pathological examination performed at CGC Genetics in 2009 were collected in a database. Our analysis included 161 cases for which information on results of prenatal examinations was available, regardless of gestational age or type of pregnancy outcome.

We believe that in all events of foetal demise, at any time during pregnancy and for every pregnancy outcome, family counselling is essential for the parents' emotional and psychological management of the situation and for their knowledge and understanding of the malformation and its recurrence risk in future pregnancies. Therefore, we have combined the foetal pathological perspective with the viewpoint of medical genetics.

Several recent studies have examined the correlation between prenatal screening/diagnosis results and autopsy findings. Like our study, most of these studies were retrospective. Overall, the studies were initiated from a neutral perspective, evaluating the value of screening methods as well as foetal autopsy. Since our cases came from a foetal pathology archive, they were reviewed from a pathological perspective.

As in most earlier studies, we classified our 161 included cases into subgroups of concordance between prenatal and autopsy results, where 95 Cases (59%) were assigned to subgroup 1 (complete concordance). This percentage is similar to results found in other studies that describe a rate of complete concordance that varied from 36-64% (4,5,6,11-15). Of our cases, 18 (11%) were classified as subgroup 2 cases (minor additional findings). This is slightly less than the 17-39% described by other studies (4,6,12,15). On the other hand, our percentage of subgroup 3 cases (relevant additional information) is slightly higher than in other studies; 29% (46 cases) versus 13-27% (4,6,16,17). This shift may be explained by our broad definition of 'relevant additional information'. For instance, cases in which placental pathology was found were assigned to subgroup 3, because this was an indication for maternal screening for haematological abnormalities.

In cases of chromosomal abnormalities or single-gene defects, the rate of concordance was the highest, because most of these abnormalities were diagnosed prenatally. Apart from chromosomal abnormalities and single-gene defects, the rate of complete concordance was highest in cases of nervous system anomalies. But for cardiac, renal and digestive malformations, autopsy provided relevant additional information (concordance subgroup 3) in more than half of the cases. Autopsy was also essential in the diagnosis of placental pathology.

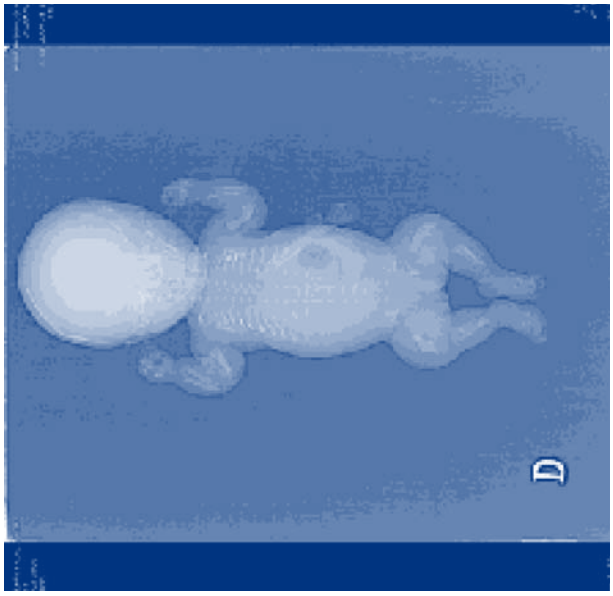
In other studies, the rate of discordance varied from 0% to 3.6% (6,11-16). Of our cases, only one was assigned to this subgroup.

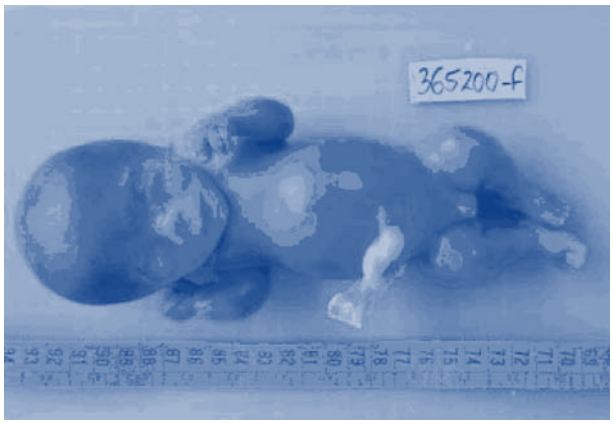
Unfortunately, information about maternal clinical or obstetric history provided by physicians was not always complete and consistent. We expected the physicians to have provided the information if it was abnormal or of particular interest for pathology or medical genetics. But it is possible that we missed relevant data. This demonstrates the importance of the provision of all relevant clinical information by physicians, on the one hand for their colleagues in pathology and medical genetics, and on the other hand for medical research. It also shows a disadvantage of the retrospective study design: if it had been a prospective study, physicians would be instructed on what clinical information to provide. Another disadvantage of the retrospective design is the possible bias in selecting and excluding the cases while already knowing the autopsy results.

In the foetal pathological examination at CGC Genetics, radiography is not standard. But in case a skeletal anomaly was identified prenatally, or was suspected at macroscopic examination, the foetus was taken to a nearby centre for radiography. (Figure 4)

Finally, our study was limited by our restricted knowledge of the Portuguese language and training in medical genetics. We avoided translation errors by asking for explanations from the laboratory staff or the pathologist. Moreover, classification of the cases into subgroups is a subjective process. We have tried to be consistent by carefully writing down our definitions and by rechecking all cases before retrieving the results. If we had any doubt, we consulted the pathologist and/or the medical geneticist.

Figure 4 - X-ray image and photograph after medical termination of the pregnancy of a foetus diagnosed with thanatophoric dysplasia. Note the short limbs, bowing of femur and humerus and hypoplastic thorax, typical for this musculoskeletal malformation.
(Pictures: Pathology laboratory, CGC Genetics, Porto, Portugal)





Conclusion and recommendations

Our results demonstrate the value of autopsy for medical genetic counselling on the recurrence risk of foetal anomalies, especially in cases not prenatally diagnosed with a genetic abnormality. Moreover, ultrasonographers, clinicians performing prenatal diagnostic tests and laboratories performing karyotyping and genetic tests benefit from autopsy results as a quality control.

Therefore, we recommend that foetal autopsy be performed in all cases of foetal demise in which no chromosomal abnormality or single-gene defect was detected by prenatal diagnosis.

Furthermore, we would like to emphasize the importance of good communication between the physician, who should provide consistent and complete clinical information, and the pathologist, who needs to give clear instructions, e.g. on how the foetus should be preserved before it arrives at the pathology laboratory.

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New Developments in Small Molecule Immunosuppressive Drugs

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Objective: **There is an ongoing search for new immunosuppressive agents that can protect organ transplantation patients while causing minimal adverse effects. In this study, we analyzed literature on new immunosuppressive drugs which could be efficacious in preventing organ rejection. The aim of this review is to select therapies with the best potential for preventing transplant rejection.**
Methods: **We searched Pubmed Database, Medline, in June 2009 with the following four substance names: AEB071, CP-690500, FTY720 and ISA 247. We only included phase II studies performed after 2006 that used one or more of the above mentioned drugs and mentioned the topic 'organ transplantation'. All other types of study were excluded.**
Results: **First trials with AEB071 showed less efficacy in preventing rejection after the 1st month. CP-690550 performed well in comparison to the immunosuppressive agents that are commonly used today. The clinical results for FTY720 showed neither a clear advantage in immunosuppression nor a reduction in adverse events. ISA247, a Cyclosporine (CsA) analog, is a potential candidate to replace the often used CsA.**
Conclusion: **After studying the adverse effects and efficacy in preventing rejection, we recommend further research on the following agents: AEB071, CP-690500 and ISA 247. FTY70 should no longer be used in organ transplantation.**

Key words - ***AEB071, CP-690550, FTY720, ISA 247, Transplantation.***

Introduction

The FDA approval of Cyclosporine in 1983 was a milestone in organ transplantation. This immunosuppressive agent prolonged graft survival and reduced mortality rates. Since then, the group of Calcineurin Inhibitors (CNIs), to which Cyclosporine belongs, has grown not only in proportion, but also in importance. These drugs are used in almost every maintenance regimen in organ transplantation. Nevertheless, CNIs have several adverse effects, with the main problem being nephrotoxicity.

In recent decades, the drug industry and clinical investigators have been searching for a replacement for nephrotoxic agents. Toxicity and chronic graft rejection are the main obstacles that need to be overcome in order to reach long-term graft survival. It is therefore crucial to develop a new drug or combination of drugs.

A possible strategy is to develop molecular drugs that target intracellular mechanisms, specifically pathways other than the Calcineurin/Cyclophillin pathway used by CNIs. Targeting specific pathways present only in T-cells seems to be the ideal approach for transplantation, due to the prominent role of T-cells in immunological reactions. At present there are several candidates for efficacious immune suppression. The aim of this review is to select therapies with the best potential in preventing transplant rejection in humans; we selected the following candidates: AEOB071, CP690550, FTY720 AND ISA 247.

Methods

Pubmed was searched in June 2009 for all relevant articles published after 2006. To be included in our study, an article had to:

- contain one or more of the following words: AEB071, CP-690500, FTY720, ISA 247,
- have organ transplantation as topic,
- be either a phase II or a phase III study,
- be written in English,
- use any study design except a review, systematic review or meta-analysis,
- be available to Erasmus MC - University Medical Center students.

In order for an article to be included, it had to meet all criteria. The data were assessed using the results of each article and combining them.

Results

Our search resulted in: 0 articles on AEB071, but we found 2 relevant abstracts from the American Transplant Congress (ATC) 2009; 15 articles on CP-690550; 9 articles on FTY720 and 0 articles on ISA247, but we found preliminary data on this drug in personal correspondence.

AEB071

AEB071 (AEB) is a new, orally administered, low molecular weight compound that blocks T-cell activation by selective inhibition of Protein Kinase C (PKC) isoforms. These PKC isoforms play a key role in signaling pathways downstream of the T-cell Receptor (first signal) and CD28 (second signal). By inhibiting PKC, it differs from the mechanism of the Calcineurin Inhibitors (CNIs). This raises the question how the efficacy and toxicity in a clinical setting will be influenced. PKC isoforms can be divided into three categories: conventional/classical, novel and atypical isoforms. These three categories have different requirements for activation. Based on in vivo studies of PKC isoenzyme-selective knockout mice, three of the isoforms, PKC-α, β and θ, have been shown to be important in T- and B-cell signaling. PKC θ seems to be the key to regulate the immune response in transplantation. Its function is largely restricted to T-lymphocytes, and it activates

the transcription factors activator protein-1 and nuclear factor κB (NFκB), which leads to Interleukin (IL)-2 production. Mice that lack PKC θ show impaired T-cell activation. AEB seems to be effective at inhibiting classical and novel PKC isotypes, including PKC θ (1). Consequently, the PKC-selective compound AEB071 is a specific inhibitor of early T-cell activation with a mechanism of action that differs from CNIs, resulting in complementary effects on proliferation and IL-2 secretion. Because it uses a different mechanism, it is thought that AEB might not have toxicities associated with Calcineurin pathway inhibition (Fig.1)

Two multicentre phase-II trials were performed between 2007 and 2009. The first study combined AEB with Mycophenolate (MPA) in de novo renal transplant patients (n=81). The protocol appeared as efficacious in preventing graft rejection as the control group, which was treated with Tacrolimus (TAC) and MPA (n = 44). After 3 months the study was prematurely terminated due to an increase in acute rejection (2). The second study combined AEB with TAC either in standard (TacS, n=76) or in reduced exposure (TacR, n=66) and compared it to a control regimen of TAC plus MPA (CO, n=74). After 3 months, both AEB regimens were converted to AEB plus MPA. The post-conversion regimen did not provide adequate efficacy and resulted in more acute rejections. For this reason, the study was prematurely terminated (3).

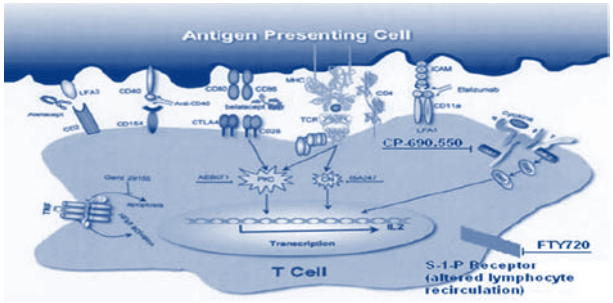

figure 1. New Targets for Immunosuppression
Adapted from Vincenti F, Kirk AD. What's next in the pipeline. Am J Transplant 2008; 8: 1972-1981.

Nevertheless both studies showed better glomerular filtration rates and thus less nephrotoxicity. AEB regimens were well tolerated and showed a level of adverse effects no higher than that of commonly used immunosuppressive agents. The first trials with AEB showed promising results within the first month, but less efficacy in preventing rejection for longer than one month. The benefit in renal function is a big step forward from the nephrotoxicity of CNI regimens, while the adverse effects seem to be the same as with other immunosuppressive agents. Clinical trials are being conducted to find the best dosage and protocol. Current trials have shown promising results with a protocol using a combination of Rapamycin and AEB.

CP-690550

Janus kinase (JAK) 3 is a tyrosine kinase associated with the common gamma chain of the IL-2 receptor family. JAK3 inhibitors block JAK3 activity and thereby block signaling downstream of gamma chain containing cytokine receptors. This causes signals emanating from certain T-cell growth factor receptors to be blunted, for example IL-2, IL-4, IL-7, IL-9, IL-15, IL-21, and impairs the development and homeostasis of immune cells. CP-690550, an orally active inhibitor of JAK3, has shown promising results in several studies. CP-690550 has a 20 to 100-fold higher affinity for JAK3 than it has for JAK1 and JAK2, and is therefore a highly specific agent for JAK3 inhibition (4).

A Phase II dose-escalation study was conducted among 28 stable renal allograft recipients. All patients received Mycophenelate Mofetil (MMF). Six patients received CP-690550 5 mg twice daily (BID), 6 patients received 15 mg BID, 10 patients received 30 mg BID, and 6 patients received a placebo. The most common adverse events were infections, but there was no apparent imbalance in the distribution of these infections between the CP-690550 groups and the placebo group. Other adverse events that did show a direct correlation to CP-690550 administration were a decrease in hemoglobin (11%), and a decrease in Natural Killer (NK) cell counts. These were apparent in the 15mg and 30mg groups only. The overall safety and tolerability observed in this study showed CP-690550 to be an acceptable alternative to current immunosuppressive agents, although the small number of patients and short duration are weaknesses of this study, and additional dose-ranging studies are warranted to better evaluate the safety of CP-690550 (5). Although CP-690550 has been shown to cause decreases in hemoglobin (up to 11%), decreases in NK cell count and other adverse events such as urinary accretions, it performed well in comparison to the immunosuppressive agents that we use today. The adverse events are much milder, and in certain cases only exist at high exposures. If the encouraging results can be confirmed in larger cohorts, CP-690550 may prove to be an excellent alternative to the current immunosuppressive agents and put an end to the high morbidity rate seen after allograft transplantation.

FTY720

The novel immunomodulator FTY720 (FTY), sometimes referred to as Fingolimod, was developed to address the clinical need for new immunosuppressive regimens which combine optimal graft protection and optimal safety. After phosphorylation in vivo, FTY acts as a high affinity agonist for the sphingosine-1-phosphate (S1P) receptor which is expressed on thymocytes and lymphocytes, resulting in internalization of the receptor. It inhibits the S1P-mediated egress of lymphocytes from lymphoid tissues, so lymphocytes cannot recirculate to graft sites and inflammatory tissues. This method of inhibition suggests that FTY may protect organ grafts without inducing generalized immunosuppression, which is different from existing immunosuppressants.

A one-year, four-arm, randomized, phase II, dose-finding study compared four groups of patients (6). Group 1 consisted of patients treated with 2.5 mg FTY with a full dose of Cyclosporine (FDC). Group 2, patients treated with 2.5 mg FTY and a reduced dose of Cyclosporine (RDC). Group 3 received 5.0 mg FTY with RDC, and group 4 received Mycophenolate Mofetil (MMF) with FDC. Patients in Group 2 receiving FTY and RDC were discontinued from the study due to increased risk of under-immunosuppression. FTY groups showed better results than the MMF group for biopsy proven acute rejection graft loss (BPAR) and death. The lowest incidence of BPAR and death was in Group 3; however, the incidence of graft loss was highest in this group. Group 1 was associated with the lowest rates of graft loss and death. Patients in de FTY treatment groups showed more drug-related adverse events than patients receiving MMF. There was a higher incidence of bradycardia and lymphopenia/leukopenia reported in Groups 1, 2 and 3. The majority of bradycardia events were mild to moderate in severity, and all were manageable. The most common adverse events across all groups were constipation, post procedural pain, nausea, peripheral oedema, vomiting and anaemia. In conclusion, this trial indicated that patients treated with a combination of 5.0 mg FTY and RDC or a combination of 2.5 mg FTY and FDC had rejection prophylaxis and tolerability comparable to patients receiving a combination of MMF and FDC.

Another phase II study made a comparison between three patient groups. Group 1 received 2.5 mg FTY, Group 2 received 5.0 mg FTY with FDC and Group 3 received MMF with FDC (7). In this comparison, Groups 1 and 2 showed a lower incidence of BPAR, graft loss and death. The incidence of acute rejection in Group 3 was higher than expected. There was a higher incidence of bradycardia in FTY-treated patients, most likely due to agonism at S1P receptors expressed in the heart atria. There was also a higher incidence of eye and respiratory adverse events in the FTY groups. The creatinine clearance decreased in FTY treated patients. The results showed that FTY 2.5 mg and 5.0 mg combined with FDC provided adequate protection from acute rejection, but were also responsible for lowered graft function and higher incidence of heart, eye and respiratory adverse events. In a phase III study, Group 1 was treated with FTY 5.0 mg once a day plus RDC (8). Group 2 received FTY 2.5 mg once a day plus FDC. Group 3 was treated with the conventional immunosuppressant MMF 1000 mg twice a day plus FDC. Because treatment with FTY 5.0 mg and RDC didn't ensure a 50% reduction of the Cyclosporine C2 levels, this group was prematurely discontinued. The incidences of first treated BPAR and first treated acute rejection were comparable in Groups 2 and 3 and lower than those observed in Group 1. The MMF showed a higher incidence of CMV infections. Reported malignancies were comparable in Groups 2 and 3, but were higher in Group 1. The FTY treated groups showed a high incidence of respiratory, cardiac and eye adverse events. This study demonstrated that FTY 2.5 mg with FDC provided comparable rejection prophylaxis to MMF with FDC. Both FTY groups were associated with decreased graft function, increased rates of macular oedema and respiratory adverse events. FTY in combination with cyclosporine provided no benefit over standard of care using MMF.

From these studies we have concluded that the clinical results of FTY are disappointing. A low dose of FTY is insufficient for adequate immunosuppression after transplantation. A higher dose of FTY has a comparable immunosuppressive potential to the standard of care. But with increasing exposure to FTY, the adverse events appeared to increase. With no clear advantage in immunosuppression and no reduction in adverse events, FTY is unfortunately not as promising as it seemed to be.

ISA 247

ISA247, or voclosporin, is a novel isomeric cyclosporine A analog mixture: a calcineurin inhibitor. The calcium dependent protein phosphatase calcineurin is responsible for the dephosphorylation of the transcriptional regulator Nuclear Factor of Activated T cells (NFAT) and is essential for NFAT's nuclear translocation and activation. NFAT activation is associated with downstream signaling for T cell activation. The chemical structure of cyclosporine A is C62H111N11O12, while that of ISA247 is C63H111N11O12. The use of CsA is commonly associated with side effects, mainly nephrotoxicity, neurotoxicity, hypertension and hyperlipidemia. It is believed that these toxic side effects are, in part, a direct result of CN inhibition. It is possible that ISA247 will cause fewer adverse events and will be more effective as an immunosuppressant (9). CsA binds to Cyclophillin, which inhibits CN. The CsA–Cyclophillin complex also affects other organs, such as the kidneys and heart. ISA247 binds to Cyclophillin, but the question remains whether the complex also influences other pathways besides the calcineurin pathway. These effects should be compared with CsA.

The abstract written by Gaber et al. mentioned that a Phase IIa study comparing ISA247 to CsA in stable renal transplant recipients demonstrated that ISA247 is efficacious and well tolerated compared to CsA (10). A new study has been started with de novo renal transplant patients where ISA247 is being compared to TAC, another calcineurin inhibitor. The investigators are looking at acute rejection in biopsy, renal function, PK/PD relationships, patient and graft survival, hypertension, hyperlipidemia and new onset of diabetes. The preliminary data have shown a rejection rate similar to that of TAC and have confirmed previous results indicating an improved safety profile.

Discussion

AEB071, a PKC inhibitor, seems to be a potential candidate to be used as an immunosuppressive drug. Its adverse effects are similar to those shown by other immunosuppressive agents and there is a significant benefit in renal function, but efficacy has not been proven beyond the first month. Ongoing trials at Erasmus MC-University Medical Center showed promising results in using a protocol combining Rapamycin with AEB071. More studies in humans are needed to support this possibility.

CP-690550, a JAK3 inhibitor, seems to be very promising. Initial in-vivo studies demonstrated more adverse effects than expected, but this appears to be a dosing problem. From personal communication, we understand that low-dosing CP-690550 protocols in combination with currently used immunosuppressive agents have shown fewer adverse effects, but also less efficacy in preventing graft rejection. More data on CP-690550 is therefore required.

Studies involving FTY had disappointing results. To reach the same level of immunosuppression, high dosage of the drug is needed. The use of high dosage FTY is associated with stronger and more adverse effects. Considering the results from several studies, FTY seems to have no potential in organ transplantation. ISA247, a cyclosporine analog, is seen as a possible replacement of CsA. Few studies have been published and not enough is known about this drug. Although it has a similar mechanism to CsA, more research needs to be done before the two can be compared.

Limitations

This systematic review yielded only a small number of studies, despite a broad search strategy. This made it impossible to draw general conclusions about the therapies. Multiple animal studies mentioned the drugs, but because were not conducted in humans, they were excluded. However, this indicates that much more data is available than we presented in this systematic review which could be used to investigate the new drugs available. Furthermore, publication bias cannot be ruled out. Negative findings are more likely to not be published and hence may be underrepresented in this systematic review.

Conclusion

In this review we investigated new immunosuppressive drugs in several phases of development. Although some showed promising results, they seemed to be less efficacious in human studies. From the data presented in this study, we can conclude that for FTY720 there seems to be no future. We recommend no further research on this drug. More human studies are needed to decide which of the three remaining drugs will be the leading candidates for use in transplantation in the years to come.

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Table 1 - overview of the articles discussed

Drug	Author and Publication Year	Pathway	Study Phase	Cohort	Regimen	Endpoint	Results
AEB071	Styrbjorn (2) (2008)	Protein Kinase C	Phase II	De novo Renal Transplants	Group 1: AEB 300mg bid + MPA + CS + BAS Group 2: TAC+ MPA + CS + BAS	Composite Efficacy Failure (CEF: BPAR, graft loss, death or loss to follow up) at 3 months	Group 1: 26 %* Group 2: 5 % Study halted after 3 months
	Budde (3) (2008)		Phase II	De novo Renal Transplants	Group 1: AEB 200mg + TacS + CS + BAS with Tac conversion to MPA after 3 months Group 2: AEB 200mg + TacR + CS + BAS with Tac conversion to MPA after 3 months Group 3: TacS + MPA + CS + BAS	CEF at 6 months	Post-conversion: Group 1: 45 %* Group 2: 34 %* Group 3: 8 % Study was prematurely halted
CP-690 550	Van Gurp (5) (2008)	JAK3 inhibitor	Phase II	De novo Renal Transplants	Group 1: CP-690 550 5 mg (BID) Group 2: CP-690 550 15 mg (BID) Group 3: CP-690 550 30 mg (BID) Group 4: Placebo	Treatment-Emergent Adverse Events	Group 1,2 and 3: 81,8% Group 4: 83,3
FTY720	Mulgaonkar (6) (2006)	S1P agonist	Phase II	De novo Renal Transplants	Group 1: FTY720 5 mg + RDC Group 2: FTY720 2.5 mg + RDC Group 3: FTY720 2.5 mg plus FDC Group 4: MMF 2–3 g plus FDC	CEF at 12 months	Group 1: 24 % Group 2: Discontinuation Group 3: 22 % Group 4: 39 %
	Tedesco-Silva (7) (2007)		Phase II	De novo Renal Transplants	Group 1: FTY720 5.0 mg + FDC Group 2: FTY720 2.5 mg + FDC Group 3: MMF + FDC	CEF at 6 months	Group 1: 24,1 %* Group 2: 28,2 % Group 3: 39,4 %
	Tedesco-Silva (8) (2006)		Phase III	De novo Renal Transplants	Group 1: FTY720 5.0 mg + RDC Group 2: FTY720 2.5 mg + FDC Group 3: MMF + FDC	CEF at 12 months	Group 1: Discontinuation Group 2: 30,8 % Group 3: 30,6 %
ISA247	Gaber (10) (2008)	Calcineurin Inhibitor	Phase II	De novo Renal Transplants	Group 1: ISA247 0.4 mg (BID) Group 2: ISA247 0.6 mg (BID) Group 3: ISA247 0.8 mg (BID) Group 4: Tacrolimus	BPAR at 6 months	Group 1: 11% Group 2: 8 % Group 3: 3 % Group 4: 9 % No Significant Difference

* Significant Result

Near Infrared Spectroscopy: an asset to the diagnosis and treatment of traumatic brain injury

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Objective: **Near infrared spectroscopy is a technique that shows great potential in measuring cerebral oxygenation. This non-invasive technique could be an asset to the diagnostic process, monitoring, and treatment of traumatic brain injury patients. The aim of this review is to determine whether this claim is tenable.**
Methods: **Medline database search was performed on January 7th 2010 (time limit 10 years) for clinical trials and epidemiologic studies with objects 'Near infrared spectroscopy' and 'craniocerebral trauma' (MeSH terms).**
Results: **An application of near infrared spectroscopy was found in the screening of neonates/infants and traumatic brain injury patients. Near infrared spectroscopy is fast and non-invasive compared to other techniques. Disadvantages include the lack of quantification, interference caused by extracranial tissue and the shallow depth penetration. A common recommendation was the need for larger studies and refinement of the technique.**
Conclusion: **The overall conclusion was that near infrared spectroscopy shows potential for clinical application in multivariable monitoring of traumatic brain injury patients. However, refinement of near infrared spectroscopy is required for more accurate measurements. This means there are reasons to continue exploring this technique for clinical use by studying larger and more heterogeneous patient populations.**

Key words
Near infrared spectroscopy, craniocerebral trauma, review

List of Abbreviations
CO = cerebral oxygenation. OD= optical density. SjO_2 = jugular venous saturation. TBI = traumatic brain injury. ScO_2 = cerebral oxygen saturation. ICP = intracranial pressure. CBV = cerebral blood volume. $rCMRO_2$ = regional cerebral oxygen metabolism. HHb = deoxygenated haemoglobin. HbO_2 = oxygenated haemoglobin. $P_{br}O_2$ = brain oxygen tension. CCO = oxidized cytochrome C. TOI = tissue oxygenation index. BFI = blood flow index. MAP = mean arterial pressure. MCAv = blood flow velocity in the middle cerebral artery. $_{PET}CO2$ = end-tidal CO_2. CVA = cerebral vascular accident.

Introduction
Every year 16.000 people in the Netherlands suffer from severe head trauma with brain injury. The effects of these types of trauma are very diverse, but every trauma has a serious impact. When brain cells are damaged, they are incapable of regeneration, thus leading to necrosis. The lack of regeneration does not necessarily cause a loss of function. The brain tissue near the traumatized area has the ability to take over the function of the lost cells. However, this process requires time, making revalidation necessary. Primary damage (e.g. bleeding, contusion, blood embolism or skull fracture) cannot be prevented and causes irreversible cell damage. Primary damage can be focal, global or diffuse.(1) Each type of damage has a different impact on the secondary damage. Secondary damage is caused by hypoxia, elevated intracranial pressure (ICP) and hypotension after the trauma.(2) As opposed to primary damage, secondary damage can be avoided and is critical for the prognosis of the patient. Therefore, monitoring variables like oxygenation, ICP and tension in the brain are crucial after severe head trauma. Near infrared spectroscopy (NIRS) is a non-invasive (3) technique that can be used to measure regional cerebral tissue oxygenation.(4) It is used in case of brain injuries such as haematomas, ruptured aneurysms and cerebral vascular accidents. The working mechanism of NIRS is based on the penetration of near infrared light through human tissue.(5) In this case, NIRS measures the optical density of the brain tissue. When the light passes through the brain, some of it will be absorbed. The rest of the light reaches the sensor, which measures how much of the light was absorbed. (6) The amount of absorption depends on the amount of oxygenated haemoglobin or deoxygenated haemoglobin (7) in the circulating blood in the cortex. (8) Normally, the difference in optical density in the hemispheres of the brain is negligible. But when intracranial damage occurs to one side of the brain, there is a measurable difference in the optical density between the two hemispheres. (3) Due to its fast and non-invasive nature, NIRS appears to be an asset to the diagnostic process and bedside monitoring of traumatic brain injury (TBI) patients. However, there have been several questions about the reliability of this technique in the past. (9) Today, NIRS is being used in medical disciplines such as traumatology (10) and cardiac surgery. (1)

The aim of this review is to determine whether near infrared spectroscopy is an asset to the diagnostic process of traumatic brain injury and the bedside monitoring of patients recovering from head trauma. Aspects discussed in this review are the following: the reliability and accuracy of NIRS for monitoring cerebral oxygenation, a comparison between near infrared spectroscopy and current techniques for monitoring TBI patients, and most importantly, the improvements needed for this technique to earn a place in today's medicine.

Methods
The online medical database PubMed was used to collect studies for the systematic review. The search terms were "Craniocerebral

Trauma", "Spectroscopy, Near-Infrared" (both MeSH terms) AND "clinical trial" (ptyp term) OR "epidemiologic studies" (MeSH term). This search was made on January 7th 2010. The date range was ten years and articles had to be written in English and available for Erasmus MC. This search produced ten articles published between 1999 and 2009 to use for this systematic review. Out of those ten articles, various characteristics and variables were extracted. The following variables were used to compare the studies: Age, type and number of patients, type of study, measurements, measurements with NIRS, advantages and disadvantages of NIRS, conclusion about NIRS and recommendations of the authors regarding the use of NIRS in a clinical setting.

Results

Table 1 summarizes the characteristics of the articles. The studies cover a total of 241 patients. The age range of the subjects was two days to eighty years. Almost all of these studies (9-17) involved some sort of trauma, such as sub-cranial haemorrhages, severe traumatic brain injury or medical complication. Only one study assessed the reliability of NIRS. (10) The studies were relatively small-scale; none included more than 50 patients, and five studies (9, 14-17) included no more than 20.

NIRS was used to asses many variables in the brain. The majority measured oxygenated haemoglobin (HbO2) and deoxygenated haemoglobin (HHb). (9, 11-14, 16, 17) Other uses were the monitoring of oxidized cytochrome C (CCO2), (13, 16) regional cerebral oxygen metabolism (CMRO2) (12) and cerebral oxygen saturation (ScO2) (15). All of these values were calculated from the measured optical density (OD).

According to the assessments of NIRS in the studies, it was most valued for its non-invasive nature. (3, 11, 13, 16, 17) Other valued attributes of NIRS were its continuous monitoring (as opposed to periodical measurements (13, 17)), its diagnostic value for most intracranial haematomas (3) and its detailed information provided regarding the oxygenation (13). Only two of the studies (12, 17) mentioned a valid sensitivity or specificity of NIRS. Not all studies were positive about the system (3, 10-14, 16, 17); the interference of extra cerebral tissues was the biggest criticism. (10, 14-16)

Another criticism is that differently oxygenated haemoglobin molecules produce different signals, and these mixed signals could result in false measurements. The lack of quantification of the

Table 1, part 1 - Characteristics of the observed studies. Each row represents one characteristic

Article	*1. Grant et al (12)*	*2. Tisdall et al (16)*	*3. Huang et al (13)*	*4. Bhambhani et al (11)*	*5. Kahraman et al (10)*
Study	Part of larger prospective study	Case study	Controlled clinical trial	Controlled clinical trial	Controlled clinical trial
Number of patients	43	8	24	38	30
Patiënt type	Neonates	Post-TBI (unconscious)	Cardio pulmonary bypass	Nondisabled moderate to severe TBI	chronic, subdural or subdural hematoma
Age	Neonates (< 15 days)	Adults (42 (20-61) years)	Infants (5-13 months)	Adults (31.5 (21-41) years)	All ages
Measurement	Presence of brain injury (MRI/ultrasound, clinically suspected)	Oxidation in cerebral cells and mitochondria by using ICP, $P_{br}O_2$ and micro dialysis MCAv	Cerebral oxygenation during different types of cardio-pulmonary bypass (by use of neuron-specific enolase, EEG, lactate)	Cerebral oxygenation and blood volume	Presence of haematoma with NIRS, CT and MRI
Measurement with NIRS	StO_2, CBV, $rCMRO_2$ (by HHb/HbO_2)	HHb, HbO_2, CCO_2 by OD	HHb, HbO_2, CCO_2 by OD	HHb, HbO_2, OD	OD in the brain
Advantage of NIRS	Good specificity and sensitivity in this small study	Non-invasive Good measurement of CCO independently	Non-invasive Continuous Provide detailed information about cerebral oxygenation No interfering with the operation	Non-invasive	
Disadvantage of NIRS	Lack of quantification Wide dispersion of results Little depth penetration	Cross-talks (mixing signals from HHb and CCO, which can lead to false results)		It is impossible to quantify oxygen	Postoperative the OD is too high because of oedema and leakage False negatives because of hair, skull and other soft tissue
Conclusion	StO_2 measurement with NIRS alone is insensitive. Measurement with StO_2, increased CBV and $rCMRO_2$ are useful (for bedside monitoring)	NIRS can help to make new treatment strategies.	NIRS is a promising method for the monitoring of cardiopulmonary bypasses	NIRS has to be refined to overcome its limitations	NIRS is reliable preoperative, but not postoperative and after craniotomy. Good diagnoses of intracranial bleeding
Recommendation	Sensitivity and specificity of NIRS is still unclear. Larger studies are necessary	Further investigation is necessary with larger studies	This study was small, with a very homogeneous group. A larger study with more heterogeneous patients was advised	If NIRS is refined, it will be useful to the rehabilitation of patients with TBI	Radiological techniques are still the gold standard. NIRS can be used as an assessment by traumatic injury to improve the outcome.

Table 1, part 2 - Characteristics of the observed studies. Each column represents one characteristic

Article	*6. Francis et al (3)*	*7. McLeod et al (14)*	*8. Watkin et al (17)*	*9. Wagner et al (9)*	*10. Ter Minassian et al (15)*
Study	Controlled clinical trial	Cohort	Cohort	Prospective functional study	Controlled trial
Number of patients	50	8	17	14	9
Patiënt type	Normal individuals	Brain injury	Preterm infants with history of hypoxia	Patients admitted at the ICU	Severe head trauma
Age	Adults (35 (17-53) years)	Adults (22-44 years)	Adults (10 (2-52) days)	Children (2 months (2 days - 11 years))	Adults
Measurement	OD difference in the brain CT: abnormalities in the brain	SjO_2, $P_{br}O_2$, Tissue oxygenation index (TOI)	Cerebral oxygenation CBF, ScO_2	Blood flow index (BFI) by indocyanine green (ICG)	MAP, ICP, MCAv, $_{PET}CO_2$, ScO_2, SjO_2
Measurement with NIRS	OD difference between various compartments of the brain and between the hemispheres	HbO_2 and HHb (to calculate TOI)	HbO_2, HHb, Hbdiff (= HbO_2 - Hb) by use of OD	HbO_2 and HHb by use of OD	ScO_2 by use of OD
Advantage of NIRS	Portable simple non-invasive Good outcome for the most intracranial hematomas	Measurement of all the blood compartments (except the large vessels)	Inexpensive non-invasive continuous Safe, sensitive enough indicator of cerebral hypoxia	Well documented accuracy Light intensity is far below the sustained intensity	
Disadvantage of NIRS	No significant OD difference for deeply located lesions, bilateral abnormalities and small hematoma	Inclusion of blood vessels, extra cerebral tissue, extra vascular blood, extra light and subarachnoid or subdural haemorrhage	Absolute quantification of Hbdiff is not yet possible	Other NIRS methods for CBF determination have failed validation with standard techniques	Influence of extra cerebral tissue and AV partitioning Variations in optical length and light scatter
Conclusion	NIRS is a simple, portable, non-invasive method to identify superficially placed, large, unilateral intracranial pathology	TOI is probably a useful asset for the measurement of cerebral oxygenation. But multivariable monitoring is better than individual measurement	NIRS' sensitivity is sufficient and able to measure the magnitude of the change. NIRS is likely to be useful in the future when measuring the cerebral oxygenation in foetus and newborn	Presently, there is no accurate NIRS-based method for absolute CBF quantification	No adequate reflection of change in SjO_2 The use of NIRS in clinical medicine is still questionable because of problems with its accuracy
Advice		TOI seems promising for monitoring cerebral oxygenation. However, more research is necessary	Technical development is necessary for refining NIRS (movement of artefacts and quantification)		

cerebral oxygenation and blood flow measured by NIRS was an important shortfall cited by three studies. (11, 12, 17) The insufficient depth penetration was another shortcoming of the method. (3, 12)

Eight studies (3, 10-14, 16, 17) (with a total of 218 patients) concluded that NIRS is a promising technique. Two other studies (9, 15) concluded that NIRS is not promising (23 patients). Most studies (9, 11-13, 16, 17) concluded that NIRS is a promising method of measuring oxygenation, but refinement is necessary for application in a clinical setting. This means tackling the shortcomings mentioned above. Two areas were mentioned where NIRS could be an asset to modern monitoring techniques: the monitoring of cerebral oxygenation (14, 17) and neurological monitoring (10, 11). One study (10) indicated that NIRS was useful preoperative, but was not reliable for postoperative use.
Recommendations referred to the need for further research, larger studies (including more patients), and improvement of the technique. (12-14, 16, 17) One study mentioned that radiological techniques are still the golden standard for monitoring. (10)

Discussion

The articles mentioned different advantages and disadvantages of NIRS. A huge advantage of NIRS is its fast and non-invasive nature. This is very important in cases where fast detection of intracranial haemorrhage is essential. Such cases include, but are not limited to, traumatic brain injury or complications during cardiac surgery. Today, NIRS is already being used during surgery (cardiac or otherwise) to monitor the brain for indications of hypoxia, such as a cerebral vascular accident. (13)

The measurement of ICP requires the insertion of transducers into the brain. The device used for this invasive technique can cause infections and haemorrhages in the brain. NIRS measurements can be done superficially, which minimizes the risk of complications. Another positive aspect of this technique is its ability to continuously monitor oxygenation in the brain. (13) This means that changes in cerebral blood flow or oxygenation are immediately detected by NIRS. This is an improvement over CT or MRI; with the latter techniques it is only possible to interpret the situation at the instant the scan is made. Yet another advantage of NIRS is that the equipment is compact and the measurement can be done while the other medical staff are doing their jobs. With optical imaging techniques like CT or MRI, the patient must be moved, and during the scan it is not possible for the medical staff to observe or treat the patient. (18) Because of its portability and quick measurements, there are possibilities for application in triage, especially when large numbers of people are involved.
NIRS thus has many advantages, but there are also limitations.

The problem most frequently mentioned is the lack of quantification. There are options to quantify the absolute haemoglobin changes, but it is not possible to measure these with NIRS. When cerebral oxygenation is measured with NIRS, an additional MRI or CT scan is necessary for quantification. With these imaging methods, the required information about the anatomy of the cortex is obtained. (18) Another possibility is to convert the measured OD with complex formulas to quantify the haemoglobin in the circulating blood. But these formulas have limitations as well; due to their complexity a small interference can lead to significant inaccuracy of the data. (17)

Interference caused by extra cranial tissue is a major pitfall in using NIRS. The differences in the anatomy of tissues that the beam has to pass through can lead to significant differences in data. (10) This can be explained by the sensitivity of the system and the complex formulas required for converting the measured OD into clinically relevant data. Another phenomenon that results in similar inaccuracy of data is the arteriovenous-partitioning of the blood (15), i.e. the difference in oxygenation which leads to a different signal. Due to the partitioning; the differently oxygenated areas emit interfering signals. The lack of depth penetration of NIRS is a problem as well, which is especially evident in the studies on haematomas. The system could not distinguish between deep lying large haematomas and superficial small haematomas. Deeply located and bilateral lesions are also difficult to identify. (3)
The majority of the studies stated that NIRS had potential, either as an addition to current monitoring and diagnostic techniques or as a stand-alone diagnostic tool. However, there are shortcomings that need to be overcome before true clinical application can be considered. NIRS is severely criticized for its inaccuracy, such as its inability to detect changes observed by other instruments (jugular vein catheter) (18) and its lack of quantification. (9) It should be noted that the negative advice about NIRS is based on the observation of just 23 patients. (9, 18)

CT scans are currently the gold standard for the diagnosis of traumatic brain injury.(7) Compared to NIRS, these scans are quite expensive - in terms of both purchase costs and variable costs. A CT scanner can cost up to 2 million dollars and $1,300 per test (20) (21). On the other hand, the NIRS system costs only about $30,000 to purchase and $300 per test (22). Consequently, the costs of using NIRS are much lower than standard trauma diagnostics. Another important benefit of NIRS is the elimination of radiation exposure. Current diagnostic devices for head trauma cases use X-rays (e.g. CT scan and radiography), which expose patients to radiation. Exposure to radiation should be avoided, due to the risk of cancer and other long term side effects. (19)

At present, NIRS alone does not provide enough information about the state of the brain. (12) A multivariable observation is required to give accurate data about cerebral blood flow (CBF), ICP and cerebral oxygenation. (14) Developments needed to make NIRS a viable clinical tool include quantification of the many possible variables it can detect, such as HbO2, HHb and CMO2. If these deficiencies can be eliminated, NIRS has the potential to be a quick and low-cost alternative for the detection of intracranial haemorrhage. (11, 14) There is no uniform conclusion about NIRS as an asset to the diagnosis and treatment of TBI. NIRS is seen as a possible application for bedside monitoring of post-TBI patients. (14, 16) However, it is also noted that NIRS is currently not accurate enough and should be refined before it can be introduced in daily clinical practice.(9, 11)

Finally, the articles we reviewed had populations of 50 or fewer patients. Larger and more heterogeneous studies are necessary to give a better view of the accuracy and other aspects of NIRS, and refinements that should be made.

Conclusion

At present, NIRS alone is not complete and accurate enough to monitor the condition of the brain. Multivariable monitoring is required, where other parameters of cerebral condition are also measured. However, because it is non-invasive and gives a fast image of intracranial haemorrhage, NIRS is a promising technique to include in the diagnostic process and bedside monitoring of TBI patients. If NIRS is refined and becomes more accurate, it can become an asset in the clinic. Larger and more heterogeneous studies are essential for the refinement of this technique.

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Treatment of neurosyphilis in HIV-infected patients.

A systematic review.

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Background and objective: **The aim of this systematic review is to provide answers to the question if HIV status has effect on the treatment of neurosyphilis and whether therapy for HIV-infected patients with neurosyphilis has to differ from non-HIV-infected.**
Methods: **We performed a literature search on PubMed on January 11th 2010. We selected articles by reading abstracts and full articles. We only included clinical trials, epidemiologic studies and case series.**
Results: **5 studies met our inclusion criteria. The decline of laboratory measures, as VDRl, CSF-VDRl and CSF-WBC, were statistically significant slower in patients infected with HIV compared with those not infected with HIV (VDRL p=0.006, CSF-VDRL p=0.02 and CSF-WBC p=0.03) Both the ceftriaxone treatment regime and the penicillin treatment regime produced comparative numbers of patients who responded to therapy, on condition that the same treatment dosage was used.**
Conclusions: **The results indicate that HIV-infected patients have a slower decline in CSF-values. There is no difference between the effect of penicillin and ceftriaxone treatment among the HIV-infected patients.**

Keywords - ***Neurosyphilis/therapy, HIV-infections, ceftriaxone, penicillin G***

Introduction

After a large decrease in the number of cases of syphilis in the past decades, the incidence of syphilis is rising again.(1) At the end of the nineties, a new epidemic of syphilis has been documented in Western-Europe and the United States.(2) Although the number of patients seems to be decreasing again, this disease is still an important infectious disease that is sexually transmitted. Many patients with newly diagnosed syphilis are also HIV-infected. These patients have a higher risk to develop neurosyphilis, an inflammation of the central nerve system. Several case reports have shown that a concurrent infection with HIV may change the natural course of neurosyphilis.(3,4,5) This might mean that patients with HIV respond different on treatment for neurosyphilis. Though, it is not known whether patients with neurosyphilis and concurrent HIV infection really respond different to treatment compared to patients without concurrent HIV-infection. But if they do, what is the best treatment for HIV-infected patients?

The purpose of this systematic review is to describe what is already known about treatment of neurosyphilis in HIV-infected patients. Do they respond different and if they do, what is the best treatment option?

Methods

Literature search

On the 11th of January 2010 we did a computerized search to identify all relevant studies published in English language in the PubMed database. We used the combination of Mediscal Subject Headings (MeSH) terms “HIV infections” and “Neurosyphilis/therapy” to perform this search.

Inclusion criteria

We only included articles that were available in free full text for the Erasmus Medical Centre. We manually searched the available articles and based on the abstracts, we included all clinical trials,

Figure 1 - Flow diagram for selection of studies

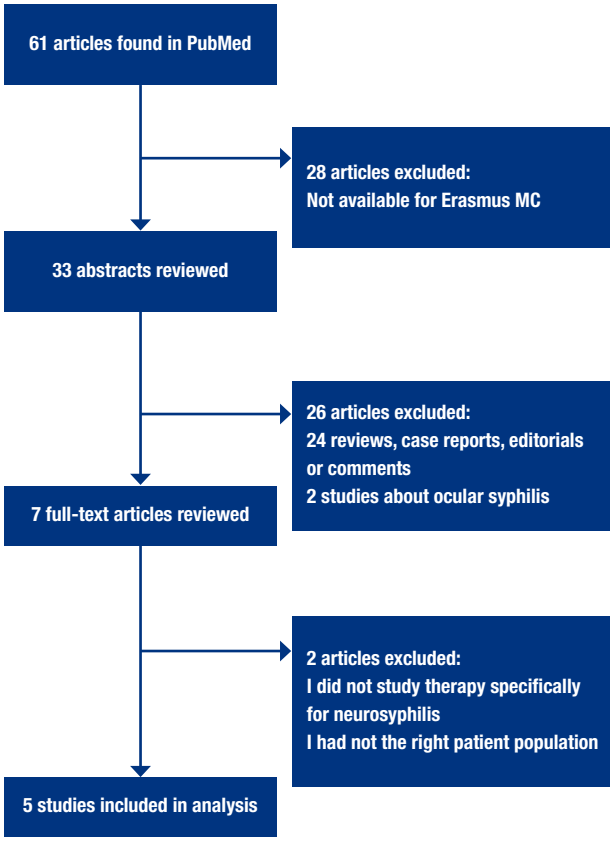

Table 1 - Included studies; IM: intramuscular, IV: intravenous, MU: million units, PPG: procaine penicillin G, APG: aqueous penicillin G, VDRL: Veneral Disease Research Laboratory test, CSF: cerebrospinal fluid, WBC: white blood cell count, RPR: rapid plasma regain; MHA-TP: microhemagglutination assay

	Location of study population	Number of patients with neurosyphilis	Intervention (number of patients)	Serum and CSF measures	Follow-up
Dowell ME et al. (1992)	Clinic, USA	9 (9 HIV)	- Ceftriaxone 1-2 g IV or IM for 10-14 consecutive days (7) or; - Benzathine penicillin, 3 times 2.4 MU (2)	RPR, VDRL, MHA-TP	> 6 months
Gordon SM et al. (1994)	Clinic, USA	11 (11 HIV)	- 18-24 MU IV APG potassium a day, in doses administered every 4 hours, for 10 days (11)	Serum VDRL, RPR, CSF-WBC, CSF protein	24 weeks
Marra CM et al. (1996)	Clinic, USA	22 (13 HIV)	- 2-4 MU IV APG every 4 hours, 10-14 days (?) or; - 2.4 MU IM PPG daily + 500 mg probenecid 4 times a day, 10-14 days (?)	Serum VDRL, CSF-VDRL, CSF-WBC, CSF protein	HIV- : 115 days HIV+: 349 days
Marra CM et al. (2000)	-	36 (36 HIV)	- Ceftriaxone 2g IV once daily for 10 days (18) or; - Penicillin G 4 MU IV every 4 hours for 10 days (18)	Serum VDRL, CSF-VDRL, CSF-WBC, CSF protein	52 weeks
Marra CM et al. (2004)	Clinics, USA	59 (46 HIV)	- highdose IV penicillin 12-24 or 18-24 MU a day, divided into 6 doses (34) - 2.4 MU IM PPG daily + 500 mg probenecid 4 times a day, 10-14 days (17) - Ceftriaxone 2g IV (7)	Serum RPR, CSF-WBC, CSF VDRL, CSF protein	12 months

epidemiologic studies and case series. Also based on the abstracts, we excluded studies about ocular syphilis.

Exclusion criteria

From the full text of the remaining articles, we independently assessed eligibility. In the end, we also excluded 1) studies about therapy that was not specifically used to treat neurosyphilis and 2) studies with patients who were not diagnosed with neurosyphilis at the start of treatment.

Results

Normalisation of cerebrospinal fluids

Study properties

Two studies of Marra CM et al.(6,7) study this phenomenon in a clinical trial that compares HIV-infected with non-HIV infected patients. Both studies define neurosyphilis as a reactive Veneral Disease Research Laboratory test (VDRL) in cerebrospinal fluid (CSF), except that the 2004 study also considers a CSF-white bloodcell count (WBC) >20 cells/μl as neurosyphilis. The fact that HIV causes a mild pleiocytosis is taken into account with this decision.

As can be seen in Table 1, the intra-musculair (IM) procaine penicillin G (PPG) and oral probenecid treatment regime was used in both studies and the other treatment regimes were different. Both studies mention - without giving any data- that there is no difference between the several specific neurosyphilis treatment regimes in the normalization of any of the laboratory measures.

Decline in laboratory measures

The 1996 study of Marra CM et al.(6) concludes that the decline in most of the laboratory measures was slower in patients infected with HIV compared with those not infected with HIV. Despite the fact that the number of patients is very small, these differences were significant for decline in serum VDRL (P=0.006), CSF-VDRL (P=0.02) and CSF WBC count (P=0.03). The decline in CSF protein was not significantly slower in HIV-infected patients. The stage of syphilis or history of syphilis did not influence this outcome.

A remarkable difference is that in the study of Marra et al. from 2004 (7), the researchers found that only the CSF-VDRL was less likely to normalize in HIV-infected subjects. Normalization of the other parameters was not significantly different.

Influence of clinical state of HIV-infection on the decline in laboratory measures

The Marra et al. 1996 (6) study makes the contention that clearance of Treponema microorganisms from the central nervous system (CNS) may be impaired by concomitant HIV-infection. To support the hypothesis that an intact immune response is -beside antibiotics- needed for cure, the writers cite some articles (8,11,12,13) who have studied this delayed decline in syphilis patients.

The 2004 study concludes that among HIV-infected subjects, those with peripheral blood CD4+ T cell counts of >200 cells/μl were more likely to normalize CSF-VDRL activity than those with peripheral blood CD4+ T cell counts of ≤200 cells/μl. The writers discuss that these results suggest that HIV-induced immune impairment contributes to the slower normalization seen in HIV-infected persons. They do not know whether decreased likelihood of normalization during the observation period is equivalent tot treatment failure, but they think the difference in treatment response in HIV-infected patients is concerning. In their opinion, future research should address this question.

However, the Dowell ME (8) et al. study concludes that there is no correlation among the CD4 cell count, the clinical state of HIV-infection and the outcome of treatment after 6 months of follow-up. Unfortunately, they do not show any data to prove this contention.

The best treatment option for neurosyphilis in HIV-infected individuals

Five articles studied the treatment options for neurosyphilis in HIV infected patients. Marra CM et al. 1996 (6) and 2004 (7) mention, without giving any data, that specific neurosyphilis treatment did not influence normalization of the CSF. Therefore we only discuss the remaining three studies in this section.

These studies of Dowell ME et al. (8) and Gordon SM et al. (9) and Marra CM et al. 2000 (10) studied which are the best treatment options for neurosyphilis in HIV-infected patients. The used treatment options were ceftriaxone and penicillin.

Laboratory measures
In all three studies a serum rapid plasma regain test (RPR), a Veneral Disease Research Laboratory test (VDRL) and a micro-hemagglutination assay (MHA-TP) or fluorescent treponemal antibody-absorption (FTA-ABS) test were done. In all three studies, a patient with a reactive CSF VDRL-test was defined as having neurosyphilis. Gordon SM et al. (9) combines this test with the clinical symptoms of patients.

The study from Dowell ME et al. (8) also regards CSF as abnormal if it contains ≥ 6 white blood cells (WBCs)/μl, or ≥ 46 mg/dL protein, or ≤ 45 mg/dL glucose.

On the other hand the study from Marra CM et al. 2000 (10) defines CSF as abnormal if it contains >20 WBC /μL or when the CSF protein concentration is >50 mg/dl.

Treatment with ceftriaxone
Dowell ME et al.[8] and Marra CM et al. 2000 (10) used ceftriaxone as treatment option for neurosyphilis. In the Dowell ME et al. (8) study 56 patients were included, but only seven patients had documented asymptomatic neurosyphilis. They were treated with ceftriaxone 1 g IV/IM (or rarely 2 g) for 10 to 14 days (Table 1). Five of these seven patients responded to therapy. There were also 2 patients with syphilis in the group that was treated with three doses of 2.4 MU benzathine penicillin, who developed a relapse. After this relapse, both patients appeared to have CSF abnormalities and were diagnosed with neurosyphilis. After this diagnosis, they were treated with ceftriaxone, 2 g daily for 14 days and they responded to this therapy.

The study from Marra CM et al. 2000 (10) included 36 patients. 18 of these patients received ceftriaxone in the same dosage as in the first study.
In the study from Marra CM et al. 2000 (10) the group treated with penicillin was compared with the group treated with ceftriaxone. Improvement in serum RPR titers was significantly more common among ceftriaxone group than among the penicillin group (8 of 10 [80%] vs. 2 of 15 [13%]; P=0.003). After correction for baseline serum RPR titers, this association between ceftriaxone treatment and decline of RPR titers became smaller, but remained significant (P=0.01).

There was only 1 ceftriaxone-treated subject with a previous episode of neurosyphilis and nearly half of the penicillin treated subjects had this history. The authors suggested that a previous episode of neurosyphilis could influence response to therapy. Serum RPR or VDRL titers declined more quickly after therapy in patients with earlier disease. Serum RPR or VDLR titers decline also more slowly in patients who have had previous episodes of syphilis.

Treatment with penicillin
Gordon SM et al. (9) and Marra CM et al. 2000 (10) studied the effect of penicillin as treatment for HIV-infected neurosyphilis patients. Both studies used a dosage of 18 to 24 MU per day of penicillin every 4 hours for 10 days. In the study from Gordon SM et al. (9) 11 HIV-infected persons were included, all were newly diagnosed with neurosyphilis. In contrast to the other two studies the patients had all neurologic manifestations. Eight patients responded to therapy: Four patients in this study had decreased serum titers (RPR) 24 weeks after treatment, and four other patients had reduction in the cerebrospinal fluid titers (VDRL).

Conclusion
The fact that the 2004 study of Marra CM et al. (7) concludes that HIV-infected subjects with peripheral blood CD4+ T cell counts of >200 cells/μl were more likely to normalize CSF-VDRL activity than those with peripheral blood CD4+ T cell counts of ≤200 cells/μl (p=0.02), might support the contention of the 1996 (6) study, which says that that clearance of CNS organisms may be impaired by concomitant HIV-infection.

Although the Dowell ME et al. (8) study concludes otherwise, they do not show any data to prove this statement, which makes this study less strong evidence.

Taking this into account, we conclude that HIV-infected patients have a slower decline in CSF-values. In patients with peripheral blood CD4+ T cell counts of ≤200 cells/μl this decline is even slower than in patients with higher CD4cell counts. Based on these findings, we can make the assumption that that the treatment of neurosyphilis in HIV-infected persons should be different, for example more intensive, than in patients without HIV, but there is in these articles not enough evidence to conclude this.
According to the results of the studies that compared different treatment options, we cannot conclude that there is a significant difference between the results of treatment with ceftriaxone or treatment with penicillin. In the same dosage both treatments have a comparative result. Though, based on the study of Marra CM et al. 2000 (10) it seems that ceftriaxone gives a significant more quickly decline in serum RPR titers than penicillin does.

Discussion
The studies included in this systematic review all had a very small study population. The Marra CM et al. study from 1996 (6) studied 22 patients; the study of Dowell ME et al. (8) only studied 7 cases of neurosyphilis and the Gordon SM et al. (9) study only 11. The Marra CM et al. study from 2004 (7) included more patients, but they had a population with little HIV uninfected patients (Table 1). The authors note that this limits their ability to detect differences between these populations. The fact that we could not find any differences between different treatment regimes could have been a result from the small study populations. Small differences between treatment options only appear in large study populations. Because of this, we think that it is necessary to study the effect of different treatment regimes in larger study populations.

There is only one study (7) that proves with data that the HIV-status of a patient influences the treatment of neurosyphilis. The normalization of the CSF is slower in patients with active HIV. As we have said in the conclusion, we can only make an assumption that HIV-status influences neurosyphilis treatment, but we think that one study is not enough evidence to prove this. Beside that, a slower normalization of the CSF does not have to mean that after a longer follow-up, treatment also had failed. It could be that it takes more time in HIV-patients to reach a normal CSF, but that success of treatment is as much in HIV-infected as in non-HIV infected patients after a longer time.

The studies of Marra CM et al. 1996 (6) and 2004 (7) mention that there is no difference in normalization of the CSF between the various treatment regimes. Unfortunately, they do not show any data to support this statement. This makes their statement less reliable and useful for our review.

Another point of discussion is the fact that there is a difference between the studies in the inclusion criteria of patients, based on the laboratory measures. There is still discussion about the cut-off of WBCcounts A result of this discussion is that the studies consider the CSF of patients as abnormal at different cut-off values. The study from Dowell ME et al. (8) regards CSF as abnormal if it contains ≥ 6 WBCs/μl, while the study from Marra CM et al. 2000 (10) defines CSF as abnormal if it contains >20 WBC /μL. Using a higher cut-off value means that the patients included in the study are more likely to really have neurosyphilis. Patients in the Dowell ME et al. (8) study with a low WBCs might

have been considered as patients with neurosyphilis, while they were only having latent syphilis. This could have influenced the results of the study.

Another difference is the inclusion of symptomatic and asymptomatic patients.

Dowell ME et al. (8) and Marra CM et al. 2000 (10) selected their patients based on labarotory measures, while in the other Gordon SM et al. (9) patients were selected based on symptomatic manifestations of neurosyphilis. In Dowell ME et al. (8) development of symptomatic manifestations of neurosyphilis is seen as failure of the ceftriaxone treatment. It could be possible that the stage of neurosyphilis could influence the effect of treatment.
The selection of treatment was not randomized in all studies.
In the study from Dowell ME et al. (8) treatment is selected by individual physicians, while in the study from Marra CM et al. (10) treatment is randomized selected. We assume that the results of the study that used randomized selection to select therapy is more reliable. In the study of Dowell ME et al. (8) treatment of ceftriaxone and penicillin was compared. It is remarkable that the patients treated with penicillin got a lower dosage of treatment. This was not enough to treat neurosyphilis. Therefore in this study it is impossible to compare the penicillin treated patients with the ceftriaxone treated patients.

During the selection of studies, we found an article (14) that reported about the effects of HIV-treatment with HAART on the cure of neurosyphilis. This study was irrelevant for the goal of our systematic review, but we think that it is a very interesting subject, to study the influence of HAART therapy and the interaction with the medication for neurosyphilis therapy.

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The effect of Botulinum Toxin Type A on Painful Bladder Syndrome.

A systematic review of the literature.

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Objective: **To systematically review and evaluate the evidence on the treatment of painful bladder syndrome (PBS) with Botulinum Toxin type A (BTA).**
Methods: **The PubMed database at the National library of Medicine was searched for English-language articles published between 2000 and 2009. We found 11 articles and selected 4 for review. We found only one randomized controlled trial. The other 3 articles were prospective cohort studies.**
Results: **In all the studies the parameters for pain score (reported by patient self-assessment using a 10-point visual analogue scale VAS), and maximum cystometric bladder capacity changed significantly after BTA treatment. Comparing Botulinum Toxin type A treatment with/to bladder distension treatment also showed a benefit of Botulinum with a p-value of 0.007. We concluded that BTA is effective on alleviating PBS symptoms for at least three to six months. BTA 100 Units appears the optimum dose for effective treatment. Knowledge about the side effects of the treatment is still limited. >>>**

Conclusions: **BTA has a positive effect on alleviating the symptoms caused by PBS. Before BTA can be introduced in clinical practice for treatment of PBS, more randomized controlled studies are needed to compare the different doses of BTA injections to the side effects they can cause.**

Keywords
botulinum toxin type A, interstitial cystitis, painful bladder syndrome, bladder distension

Introduction

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating chronic disease of unknown etiology characterized by frequency, nocturia and suprapubic pain related to bladder filling (1). Current treatments are usually unsuccessful in completely eradicating bladder pain and increasing bladder capacity (2). At present, bladder distension (BD) is the most common treatment for IC/PBS, but the therapeutic duration is short (3).

Recently, Botulinum Toxin type A (BTA) was introduced for the treatment of overactive bladder syndrome and several lower urinary tract dysfunctions (4). Although the efficacy of BTA has been widely reported (5,6), there have only been a few studies using BTA for the treatment of IC/PBS (7-9). Research had shown that BTA can inhibit not only the release of acetylcholine and norepinephrine, but also that of ATP and calcitonin gene-related peptide from the detrusor muscle and urothelium (10-12). The effect of BTA is likely to be mediated by blocking of the release of several neurotransmitters involved in afferent nociceptive transmission (13). The possible complications associated with BTA injection are generalized muscle weakness, difficult urination, transient urinary retention and urinary tract infection (UTI) (1).

In clinical experiments, BTA has been shown to decrease detrusor overactivity, bladder sensation and visceral pain in chronic inflammatory disease (14). All these results suggest that BTA can alleviate the symptoms caused by IC/PBS. We reviewed the literature to answer the following research questions for the treatment of painful bladder syndrome with Botulinum Toxin type A: Does Botulinum Toxin type A have a positive effect on alleviating the symptoms caused by PBS? How long do the positive effects of the treatment last?

Methods

We searched the PubMed database at the National library of Medicine for English-language articles published between January 2000 and December 2009. We used the following Medical Subject Headings: Interstitial Cystitis AND Botulinum Toxin type A. We selected Restrict Search to Major Topic Headings only. We initially intended to only include randomized controlled trials in the systematic review.

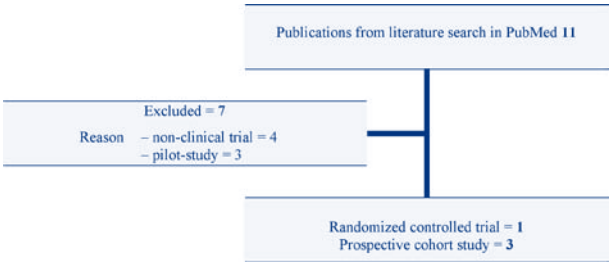
Results

Description of studies

Our PubMed search yielded 11 publications (Fig.1). These publications described 3 pilot-studies, 4 non-clinical trials, 3 prospective cohort studies and 1 randomized controlled trial.

We initially based our selection on studies reported as randomized controlled trials, but the PubMed search produced only 1 randomized controlled trial. Therefore we also included the 3 prospective cohort studies, because the level of evidence in these studies was higher than in the other articles we found (Attachment 1). We therefore included 4 publications for review. The other articles were excluded.

Figure 1 - Metrics of literature search



The patients studied in all trials were very similar. Only patients who were diagnosed with painful bladder syndrome were enrolled in the studies. The diagnosis of PBS was established according to the characteristic symptoms of PBS, including urgency, frequency and suprapubic pain, and according to cytoscopic findings. All patients were examined thoroughly according to the National Institute of Diabetes, and Digestive and Kidney disease (NIDDK) criteria (1). In each study patients were only enrolled if previous conventional treatment had failed.

The characteristics of the included studies are shown in Table 1. The studies were published between 2004 and 2009. The population size varied from 13 to 67. Patients were predominantly female and usually middle-aged. The follow-up time was at least 3 months.

The effect of Botulinum Toxin type A

Table 2 presents the four trials that confirmed the positive effect of Botulinum on PBS. The four trials used different parameters to measure the effect of BTA on the bladder. Table 2 shows the parameters that were used in all studies; daytime and nighttime frequency (n), pain score reported by patient self-assessment using a 10-point visual analogue scale (VAS), maximum cystometric bladder capacity (ml) and urge of voiding (ml). All studies concluded that BTA provided long-term pain relief and increased the maximum cystometric bladder capacity.

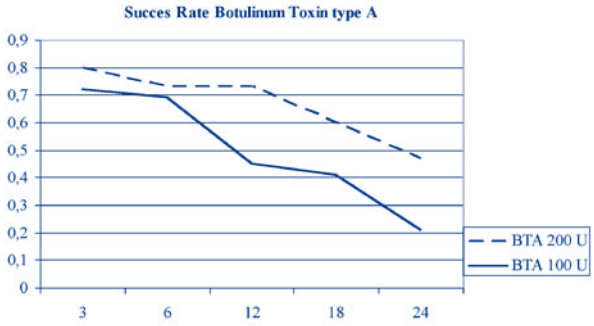
Botulinum Toxin type A and bladder distension

The effect of bladder distension (BD) in combination with BTA was reported in the study of Kuo et al. 2009, which was the only randomized controlled trial (1). The control group underwent bladder distension. The other two groups received injections of either 100 U or 200 U Botulinum Toxin type A. At two weeks after the injections the patients also underwent bladder distension. This study not only showed an effect of Botulinum toxin type A on PBS, but also compared the effect to bladder distension only. Table 2 shows the increase in maximum cystometric bladder capacity in the two Botulinum groups and in the bladder distension group. The increase in the BTA-100 group was 26%, in the BTA-200 group it was 63% and in the bladder distention group only 4%. This showed that there was a significant increase (p-value of 0.007) of the maximum cystometric bladder capacity in the patients who received Botulinum Toxin type A compared with the control group, which only received bladder distention.

The beneficial effect in time-line

Three studies reported about the effect of Botulinum Toxin type A on painful bladder syndrome over time. Before BTA injection, baseline data were collected and compared to the results of follow-up data. The success rate was defined as the number of patients who had reduced PBS symptoms. For BTA 100 U, Kuo et al. (1) reported a 72% success rates with a therapeutic duration of 3 months. The therapeutic effect decreased to 69% at 6 months and to 45% after 12 months (Fig. 2). Giannantoni et al. (15) found a 86% success rate in the first three months. The beneficial effect decreased to 30% after 5 months, and there was no effect after 12 months. At 1-year follow-up, symptoms recurred in all patients. Smith et al. 2004 (7) noted 69% improvement of symptoms, which lasted for 3.72 months.

Figure 2 - Kuo et al. 2009 [1] The effect of BTA in months



Units of Botulinum Toxin type A required

An important question is: what amount of BTA is needed for an optimal result? The ideal is to achieve the best effect for the patient at the lowest treatment costs. Note that a single dose of 100U costs €300, and 200U costs twice as much. Two of the four studies compared BTA 100 U and BTA 200 U. After 3 months both groups achieved a significant increase in cystometric bladder capacity and a significant decrease in pain VAS (Table 2). However, compared to each other there was no statistically significant improvement (Table 2 – Kuo et al. (1)). The adverse events after treatment with BTA 100 U or BTA 200 U were different. Difficult urination (dysuria) and a large postvoid residual volume were problems which occurred in both groups. In the BTA 100U group occurred these adverse events in 17% respectively 50% of the patients. In the BTA 200U group occurred these adverse events in 80% respectively 100% of the patients. (1,16) Knowledge about the side effects of the treatment is still limited.

Discussion

This systematic review, based on 1 randomized controlled trial and 3 prospective cohort studies, evaluated the effect of Botulinum Toxin type A on patients with Painful Bladder Syndrome. We concluded that BTA has a positive effect on alleviating the symptoms caused by PBS. However, this effect is optimal for only 3 to 6 months, and after that the effect decreases. An important question now is whether this effect of BTA is long enough to consider it as a good treatment for PBS. The treatment is invasive and must be repeated every 3 to 6 months. We have to consider whether the benefits of the treatment outweigh its disadvantages.

Decreases in daytime and nighttime frequency, VAS pain score and the urge of voiding were observed. Also, an increase in the maximum cystometric bladder capacity occurred in all 4 studies. These positive effects continued for at least 3 to 6 months, as shown in Figure 2. After that, the effect of BTA rapidly decreased to where almost no beneficial effects were remained. We can therefore conclude that BTA has a limited effect on PBS. This is why the treatment of PBS with BTA should probably be repeated every 3-6 months to maintain the benefits. The priority for further study is to determine whether it is possible and beneficial to inject patients every 6 months, keeping in mind the high cost of BTA and the potential side effects of anaesthesia.

Furthermore, we conclude that there are negative side effects when using both dosages (200 units as well as 100 units) of BTA, but that side effects were predominantly present in the group using the higher dosage. Difficult urination (dysuria) and a large postvoid residual volume were problems which occurred in both groups. In the BTA 100U group occurred these adverse events in 17% respectively 50% of the patients. In the BTA 200U group occurred these adverse events in 80% respectively 100% of the patients (1,16). As a consequence, catheterization was sometimes needed in these patients. However, there was no significant difference between the effect of 100U and that of 200U BTA. Considering the comparable treatment results, the costs of BTA and the side effects, using 100 units of BTA seems more effective in the treatment of PBS and should be preferred to 200 U BTA.

Conventional bladder distension is still the most common treatment for PBS, but the therapeutic duration is short. The randomized controlled trial of Kuo et al. showed that additional BTA injections improved the results. Maximum bladder capacity was significantly better (p-value of 0.007) when the bladder distention treatment was combined with Botulinum Toxin type A group.

This conclusion is important for future research on the treatment of PBS. Current treatments are usually unsuccessful in eradicating bladder pain and increasing bladder capacity (2). No intravesical therapy has shown long-term effectiveness (17-19). BTA has shown positive effects on eradicating the symptoms of PBS and may therefore be a good alternative treatment. However, repeated injections are needed for the effective control of bladder pain and urgency symptoms (15). Potentially, Botulinum Toxin type A could lead to long term effectiveness in managing PBS symptoms.

To our knowledge this is the first systematic review on PBS in combination with BTA. BTA appears to be successful as a treatment for PBS. But the therapeutic duration and the long term effects have not been fully determined. Important questions for future research are the following: Will repeating the BTA injections increase the duration of the symptom-free period? Are there negative side effects from repeated injections? What is the optimum dose of Botulinum Toxin type A for the treatment of IC/PBS? Do the benefits outweigh the relatively high costs of BTA treatment? To answer these questions randomized controlled studies are needed, which compare Botulinum Toxin type A injections to other therapies and controls.

After searching PubMed with MeSH terms for publications, we found only 4 useful trials. Because PBS in combination with BTA is a very recent development, not many studies have been published. Only 1 study was a randomized controlled trial. This could be a shortcoming in our study, as only this study was a level 1b study, and the rest were level 3 studies. These levels are explained Attachment 1. Also, the latter three studies used very small populations, ranging from 13 to 19 patients. This may suggest that the doctor who treated some of his patients with BTA published the positive findings before comparing the results with a control group. The results can be questioned as population bias can not be excluded. The level 1b study is more representative because is was randomized, and there should be no population bias, but we do not know this for sure because the article does not describe

how they randomized their patients. Also, the population was much larger, and the results after the intervention were compared to a control group.

Although we only found 4 studies that we could use, the distribution of patients in the 4 studies seems to reflect the clinical situation in different areas of the world. So the outcomes may be relevant for people from different continents.

In summary, Botulinum Toxin type A has a positive effect on painful bladder syndrome. The beneficial effect continues for at least 3 to 6 months. The most effective treatment seems to be BTA 100 U in combination with bladder distension. Despite these findings, many questions remain before BTA can be introduced in clinical practice for treatment of PBS. To address these questions, randomized controlled studies are needed which compare different doses of BTA injections to the side effects they can cause.

Table 1 - Studies included in the systematic review

Study	Study type	Level of evidence	Number of patients	Population with PBS	Age (SD)	Follow-up
Smith et al. (2004)	Prospective Cohort	Level 3	15 (12 female,	Poland and USA	52.5 ± 13.4	3 months
[7]	Study		3 male)			
Liu et al. (2007)	Prospective Cohort	Level 3	19 (14 female,	Taiwan	37.0 female	3 months
[16]	Study		5 male)		41.0 male	
					No SD	
					available	
Giannantoni et al. (2008)	Prospective Cohort	Level 3	13 (female)	Italian	58.0 ± 9.9	12 months
[15]	Study					
Kuo et al. (2009)	Randomized Control-	Level 1B	67 (56 female,	Taiwan and USA	48.6 ± 14.2	24 months
[1]	led		11 male)			
	Trial, Prospective,					
	Blinded					
	Comperative Study					

Table 2 - Changes of voiding diary, pain VAS, maximum cystometric bladder capacity and urge at baseline and 3 months

Study	Daytime frequency, n	Nighttime frequency, n	Pain VAS	Cystometric bladder capacity, ml	Urge voiding, ml
Smith et al. (2004)	Botulinum 100 U				
[7]					
Baseline	16.0 ± 2.94	7.6 ± 1.60	7.8 ± 1.92	159 ± 39.90	74.6 ± 11.8
3 months	9.0 ± 2.78	4.2 ± 1.52	1.6 ± 0.93	250 ± 46.10	118 ± 20.4
P-value	P < 0.01	P < 0.01	P < 0.01	P < 0.01	P < 0.01
Liu et al. (2007)	Botulinum 100 U –	200 U only responders			
[16]					
Baseline	12.6 ± 4.3	-	5.16 ± 2.09	276.2 ± 94.8	251.3 ± 89.2
3 months	8.8 ± 2.5	-	2.53 ± 1.43	372.1 ± 149.3	321.1 ± 96.9
P-value	P = 0.001	-	P < 0.0001	P = 0.009	P = 0.029
Giannantoni et al. (2008)	Botulinum 200 U				
[15]					
Baseline	15.2 ± 3.9	5.5 ± 1.5	9.4 ± 0.9	256.4 ± 33.5	< 150
3 months	8.7 ± 2.1	2.4 ± 1.7	6.8 ± 1.7	325.5 ± 50.0	-
P-value	P < 0.01	P < 0.05	P < 0.01	P < 0.01	-
Kuo et al. (2009)	Botulinum 100 U				
[1]					
Baseline	13.0 ± 4.69	3.41 ± 2.16	4.83 ± 2.21	308.5 ± 135.0	263.9 ± 89.5
3 months	9.72 ± 4.03	2.59 ± 1.97	2.97 ± 1.99	388.0 ± 126.8	301.8 ± 72.8
P-value	P > 0.05	P > 0.05	P < 0.01	P < 0.01	P > 0.05
	Botulinum 200 U				
Baseline	14.2 ± 5.44	6.33 ± 6.96	5.47 ± 2.1	250.5 ± 86.7	237.3 ± 70.1
3 months	9.40 ± 3.22	3.13 ± 2.47	2.47 ± 2.1	406.9 ± 178.6	319.1 ± 118.2
P-value	P < 0.05	P > 0.05	P < 0.01	P < 0.01	P < 0.01
	Bladder distention				
Baseline			4.30 ± 2.60	280.2 ± 100.8	
3 months			3.52 ± 3.07	292.0 ± 99.5	
P-value			P > 0.05	P > 0.05	

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Attachment 1 (20) - LEVELS OF EVIDENCE

Level	Type of evidence
1a	Evidence obtained from meta-analysis of randomized trials
1b	Evidence obtained from at least one randomized trial
2a	Evidence obtained from one well-designed controlled study without randomisation
2b	Evidence obtained from at least one other type of well-designed quasi-experimental study
3	Evidence obtained from well-designed non-experimental studies, such as comparative studies, correlation studies and case reports
4	Evidence obtained from expert committee reports or opinions or clinical experience of respected authorities

GRADES OF GUIDELINE RECOMMENDATIONS

Grade	Nature of recommendations
A	Based on clinical studies of good quality and consistency addressing the specific recommendations and including at least one randomized trial
B	Based on well-conducted clinical studies, but without randomized clinical trials
C	Made despite the absence of directly applicable clinical studies of good quality

Side-Effects of Sirolimus-Eluting Stents on Coronary Endothelial Function

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Objectives: **The aim of the present study was to evaluate the side-effects of sirolimus-eluting stents (SES) on the coronary endothelial vasomotor function.**
Background: **The SES is a widely used Drug-Eluting Stent (DES) coated with sirolimus, which is used for coronary revascularization interventions. Compared with bare-metal stents (BMS), it reduces stent restenosis.**
Methods: **We searched the PubMed Database for studies on vascular endothelial function in the coronary arteries from patients with BMS and SES.**
Results: **Comparing BMS and SES, there is a major difference in vasomotion. Vasoconstriction can be observed in both groups, but proximal and distal to the stent there is more vasoconstriction in the SES group .**
Conclusions: **The present study implies endothelial dysfunction as long-term side-effect of SES. Compared to BMS, the paradoxical vasoconstriction at the SES is more prominent in the segment that is distal to the stent than in the proximal segment.**

Keywords
sirolimus-eluting stent, bare-metal stent, side-effects, coronary endothelial dysfunction

Introduction
The sirolimus-eluting stent (SES) is a drug eluting stent (DES) coated with sirolimus. The SES is widely used as a DES for coronary revascularization interventions because it reduces stent restenosis compared to bare-metal stents (BMS)(1,3). Sirolimus is a macrolide antifungal agent with antiproliferative and immunosuppressant properties. It inhibits endothelial cell proliferation via cell cycle arrest in the late G1-phase(1,3).

Recent studies have raised concerns about endothelial dysfunction of the coronary vasculature adjacent to SES. This impairment could possibly lead to an inadequate increase in coronary blood flow in response to increased metabolic demand, thereby causing myocardial ischemia.

One way to induce increased metabolic demand is to administer Acetylcholine (Ach). Normally the effect of Ach on healthy endothelium is vasodilatation. However, if the vascular endothelium is damaged, paradoxical vasoconstriction may occur. In this review we therefore assessed this possible side-effect. We reviewed the literature to date which examined the effects of SES on coronary endothelial vasomotor function.
Vasomotion refers to the spontaneous oscillations in vascular tone. This change of vascular tone is generated from factors within the vascular wall. This means that it is independent of heartbeat, innervation or respiration.

Methods
Literature Search
We looked only at the vascular endothelial function in the coronary arteries from patients with either BMS or SES. Therefore, on January 12th 2010 we searched the PubMed database: ("2005/01/01"(PDAT) : "3000"(PDAT)) AND "Stents"(MeSH) AND "Endothelium, Vascular"(Mesh) AND "Sirolimus"(Mesh) AND ("Endothelium/physiopathology"(MeSH Terms) OR "Vasomotor System/physiopathology"(Mesh) OR "Endothelial Dysfunction"(All Fields)) AND "Bare-Metal"(All Fields). We found 10 articles, from which we excluded 2 with the following criteria: Prinzmetal's Angina Pectoris or study design Review. The articles we included had to be available from the Medical Library of the Erasmus MC Rotterdam. As shown in Table 1, we identified 8 clinical trials, with a total of 267 participants.

Endothelial function evaluation
The articles reported different types of stimulus to evaluate the endothelium-dependent vasomotor response. Most studies induced vasodilatation with an intracoronary infusion using differing doses of Ach. One study used the Cold Pressure Test. In normal healthy individuals, exposure to cold by immersing a foot in ice water prompts a metabolically induced increase in MBF that can be associated with a flow-mediated, and thus an endothelium dependent, coronary vasodilation(9,10). A bicycle exercise test was used in one other study (see Table 1). However, all of these tests are assumed to release nitric oxide and thereby induce endothelium dependent vasodilatation, which made comparison between the studies possible. Usually, vasodilatation was measured by Quantitative Coronary Angiography (QCA) proximal and distal to the stented region (see Table 1). However, Horigome et al.(4) evaluated distal vasodilatation by measuring myocardial blood flow (MBF) with Positron Emission Tomography (PET).

Baseline characteristics
Table 2 shows the patient characteristics for the BMS and SES-groups in all studies. These patient numbers are not always identical to the total number of patients, because there were patients who were lost in follow-up during the study.

Exclusion criteria
We excluded patients with Zotarolimus-(ZES) and Paclitaxel-Eluting Stents (PES), because these types of stent were beyond the scope of the review.

Table 1 - Study characteristics

Study	Study Design	Number of pts	BMS vs. SES	Pts criteria	Endothelial stimulus	Endoth. function evaluation	Prox/dist segment iin mm	Follow-up in months
Horigome et al.(4)	CT	14	7/7	SAP or ACS	CPT	MBF	/	1
Kim et al.(5)	CT	55	20/10	SAP	Ach	QCA	0-5	6
Kim et al.(6)	CT	75	10/39	/	Ach	QCA	0-5	6
Shin et al.(7)	CT	22	5/9	Excl. AMI	Ach	QCA	5-10	6-9
Obata et al.(8)	CT	29	16/13	AMI	Ach	QCA	15-25	0.5
Fuke et al.(1)	CT	35	12/21	SAP	Ach	QCA	0-5	6
Hofma et al.(2)	CT	12	7/5	Excl. AMI	Ach	QCA	2-17	6
Togni et al.(3)	CT	25	11/14	CAD	Bicycle exercise	QCA	5-10	6±1

CPT=Cold Pressure Test, Ach=Acetylcholine, MBF=Myocardial Blood Flow, QCA=Quantitative Coronary Angiography, pts=patients, CT=Clinical Trial, /=no data available
SAP=Stable Angina Pectoris ACS=Acute Coronary Syndrome AMI=Acute Myocardial Infarction Excl.=Exclusion Criteria

Table 2 - Baseline Characteristics

Study	Age BMS	Age SES	Male BMS %	Male SES %	HT BMS %	HT SES %	Smoking BMS %	Smoking SES %	DM BMS %	DM SES %
Horigome et al.(4)	67.7 ± 8,7	57.1 ± 8.3	80	80	70	70	/	/	50	50
Kim et al.(5)	59.7 ± 9,9	62.4 ± 8.8	56	47	30	26	20	21	20	21
Kim et al.(6)	60.7 ±8.8	63.0 ±9.5	60.0	56.4	50.0	41.0	20.0	20.5	20.0	17.9
Shin et al.(7)	61.2 ± 5.3	62.8 ± 5.9	60.0	55.5	80	77.7	40	66.6	40	55.5
Obata et al.(8)	65.1 ±12.4	63.6 ±10.1	75.0	69.2	68.8	69.2	56.3	46.2	50.0	38.5
Fuke et al.(1)	73 ± 9	70 ± 9	75	91	/	/	/	/	/	/
Hofma et al.(2)	65 ± 9	52 ± 5	80	43	40	43	40	43	0	14
Togni et al.(3)	57 ± 12	54 ± 13	82	85	55	43	64	50	9	7

BMS=Bare Metal Stent Group, SES=Sirolimus Eluting Stent Group, HT=hypertension, /=no data available

Results

We compared the vasomotion distal and proximal to the stented region. The outcomes are shown in Table 3. For the studies that used Ach during follow up, we only examined the results of the maximum doses, because those doses were most similar.

Study outcomes

Table 3. Study outcomes

Study	BMS Proximal	Distal	SES Proximal	Distal
Horigome et al.(4)	/	+58*	/	+38*
Kim et al.(5)	-8.99•	-13.2•	-22.8•	-69.9•
Kim et al.(6)	-6.23	-21.6	-24.7	-70.9
Shin et al.(7)	-16.9	-16.9	-20.3	-19.4
Obata et al.(8)	/	-5,0	/	-147
Fuke et al.(1)	+5.0	+5.1	-14.1	-17.5
Hofma et al.(2)	/	-2	/	-32
Togni et al.(3)	+6.8	+7.7	-6.8	-9.2

Mean coronary segment diameter change in percentages from baseline after endothelium-dependent vasomotion. 'Positive numbers'=vasodilatation, /=no data available *=Increase in MBF •=adjusted means for differences among SES and BMS groups according to stent length and late los

In the trial of Horigome et al.(4), in Japan, 14 patients successively received Percutaneous Coronary Intervention (PCI). The patients were classified into 2 groups depending on the PCI performed: the conventional PCI group and the SES group. Coronary endothelial function was wdefined as the percent increase in the MBF during CPT. This MBF was assessed within 1 month; it was significantly lower in the SES group ($p<0.05$).

The purpose of the trial Kim et al.(5) (in South Korea) was to compare endothelial dysfunction proximal and distal to the stent in patients with ZES compared to those with SES. The 50 patients in the trial were randomly assigned treatments: 20 ZES, 20 SES and 10 BMS. The endothelial function was assessed before intervention and after 6 months follow-up. Vasoconstriction to Ach was more prominent in the distal segments in the SES group compared to the BMS group ($p<0.001$).

The other study of Kim et al.(6) (also in South Korea) was designed to investigate whether endothelial dysfunction is related to DES implantation at 6 months after stenting. They included 75 patients, of which 39 received a SES, 36 received a PES and 10 received a BMS. Vascular responses to Ach were measured by QCA proximal and distal to the stent. Vasoconstriction to Ach was more frequently present in segments distal to stents in the SES group compared with those in the BMS group ($p<0.001$). Vasoconstriction proximal to the stent was not significant ($p=0.09$).
In the clinical trial of Shin et al.(7) (in South Korea) assessed coronary endothelial function after DES implantation compared to BMS at 6 to 9 months follow-up. Five patients were assigned to the BMS group and 17 to the DES group (9 SES and 8 PES). Endothelium-dependent vasomotion was determined after intracoronary infusion of acetylcholine. Proximal to the stented segment, the vasoconstriction in the SES group was not significantly different than the BMS group ($p=0.07$). However, there was significantly more vasoconstriction distal to the stented segment in the SES group ($p<0.05$).

The study of Obata et al.(8) (in Japan) examined whether SES implantation affected endothelial vasomotor dysfunction in coronary arteries in Acute Myocardial Infarction (AMI). In this study, 13 Patients received a SES and 16 patients received a BMS. Two weeks after intervention, endothelial dysfunction was assessed by QCA following Ach infusion in the Left Anterior Descending coronary artery (LAD) distal to the stent. They found a difference in vasomotion distal to the stented segment: there was significantly more vasoconstriction in the SES group ($p<0.005$).

The trial conducted by Fuke et al.(1) (in Japan) included 21 patients treated with SES and 12 treated with BMS. Endothelium-dependent vasomotor function proximal and distal to the stent was evaluated after 6 months by QCA, using intracoronary Ach infusion. They found significant vasoconstriction both proximal and distal to the stent ($p<0.05$).

The Erasmus MC study of Hofma et al.(2) (in the Netherlands) assessed local epicardial endothelial function 6 months after SES or BMS implantation. In 12 Patients (7 SES, 5 BMS) endothelium-dependent vasomotion distal to the stented region was assessed with QCA immediately after the procedure and at 6 months follow-up, after intracoronary infusion of Ach. Significant vasoconstriction was found in the SES group ($p=0.03$).

The study of Togni et al.(3) (in Switzerland) assessed coronary vasomotor response to exercise after SES implantation. They used 11 patients in the BMS-group and 14 in the SES-group with a follow-up at 5-7 months. Coronary vasomotion was evaluated with biplane QCA at rest and during supine bicycle exercise. The reference vessel showed exercise-induced vasodilatation in both groups. There was exercise-induced vasoconstriction of the proximal and distal vessel segments adjacent to SES ($p<0.001$ compared to corresponding segments of controls).

Summary

Comparing BMS and SES, we found a major difference in vasomotion. In both groups vasoconstriction can be observed. Four of the studies in Table 3 showed more vasoconstriction in the SES group both proximal and distal to the stent. Three of these studies only measured distal change in vasomotion, but there was also more vasoconstriction in the SES group.

Discussion

Acetylcholine dilates normal coronary artery segments by promoting the synthesis and release of nitric oxide in the endothelium (endothelium-derived nitric oxide)(11). In contrast, in segments where endothelial damage is present, acetylcholine constricts coronary arteries because of disturbed nitric oxide synthesis and release(12). All the studies found that SES implantation induces coronary endothelial dysfunction. Therefore, the present study suggests that endothelial dysfunction is a side-effect of SES implantation. Compared to BMS, this effect is more prominent in the segment that is distal (rather than proximal) to the stent. The increased vasoconstriction distal is perhaps due to the blood flow to distal.

Study limitations

Horigome et al.(4) differed from the other studies because they used the CPT as an endothelium-dependent vasomotor test. They also assessed the MBF, while the other studies used QCA to evaluate the diameter of the coronary vessel. This made it difficult to compare Horigome et al.(4) with the rest, because MBF tells us more about the microcirculation and QCA tells us only something about the conduit arteries.

The inclusion and exclusion criteria were not comparable in every study. Most of the studies excluded patients with AMI or they included patients with Stable Angina Pectoris (SAP), except Obata et al.(8). In that study they excluded patients with AMI. These patients have more damage due to primary disease, so it is difficult to distinguish between the side-effects from SES or BMS and the effects of AMI itself.

The Ach doses were very different in each study. Some studies lacked information about the concentration that was given and they often didn't describe the duration of the Ach infusion. As a result, making a comparison was difficult. We therefore choose the highest dose per study to obtain the maximum response. The results were similar irrespective of the Ach doses. This could indicate that the different doses are not very important for making a good comparison between the studies.

Despite the fact that we used 8 studies for this systematic review, a total of only 267 patients were enrolled. Of all the studies, the largest BMS group had 20 patients (Kim et al.(5)) and the largest SES group had 39 patients (Kim et al.(6)). Therefore, to overcome the limitations of statistics on small sample sizes, the results should be confirmed in larger studies.

Six studies had a follow-up with a minimum of 6 months. However, 2 studies had a maximum follow-up of only 1 month (see Table 1). Such a brief follow-up is dubious, because stent implantation itself results in endothelial injury. This injury should be healed before the effect of Sirolimus on the coronary endothelium can be assessed, since it can influence the results.

Conclusions

All the studies reported coronary endothelial dysfunction after SES implantation in humans. Therefore, the present study suggests endothelial dysfunction as a long-term side-effect of SES. Compared to BMS, the paradoxical vasoconstriction is more prominent in the segment distal to the stent than in the proximal segment. This suggests that Sirolimus affects the vascular endothelium in alignment with the bloodflow to distal.

Based on the results, we do not recommend implanting SES in patients with coronary disease.

Regarding future research, it may be useful to repeat the studies with a longer follow-up, so that the long-term clinical side-effects of SES can be assessed more credibly.

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Review

The Effectiveness of Percutaneous Coronary Intervention plus Optimal Medical Therapy versus Optimal Medical Therapy alone in Stable Coronary Artery Disease.

A systematic Review.

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Objective: **To reduce the risk of cardiovascular events, it is unclear whether percutaneous coronary intervention (PCI) plus optimal medical therapy (OMT) in patients with stable coronary heart disease is superior to optimal medical therapy alone. The aim of this systematic review was to find out which therapy is most effective in reducing cardiac complications in patients with stable coronary artery disease.**
Methods: **A literature search for randomized controlled trials was conducted via PubMed database on 17th January, 2010. We performed a systematic search on PubMed using combinations MeSH_terms. We limited our search to human studies and accepted only studies written in English. From the 92 articles found, we made a final selection based on the titles and abstracts. Studies were included if they reported quantifiable information about the effectiveness of coronary intervention (revascularization) in patients with stable angina pectoris.**
Results: **A total of 3 randomized controlled trials met our inclusion criteria. In these studies, 1845 patients were randomly assigned to PCI+ medical therapy and 1844 to medical therapy alone. During follow-up (between 2.7 and 4.6-years), there was no significant difference in the cumulative death rate between PCI+ medical therapy and medical therapy alone (2.9% vs 3.9%; hazard ratio, 0.87; 95% CI, 0.278 to 2.604). However, the cumulative risk of death plus acute coronary syndrome was significantly lower in PCI+ medical therapy (14.9% vs. 7.9%; hazard ratio, 0.47; 95% CI, 0.243 to 0.881).**
Conclusion: **The patients with chronic coronary disease may expect relief from angina (or stabilization of disease) whether they are treated with PCI plus optimal medical therapy or with optimal medical therapy alone. However, an initial strategy of PCI added to optimal medical therapy relieved angina and improved self-assessed health status to a greater extent than an initial strategy of optimal medical therapy alone for approximately 24 months. A greater benefit from PCI was observed in patients with more severe and more frequent angina. With longer duration of follow-up, the differences between the two treatment strategies diminished.**

Abbreviations and acronyms

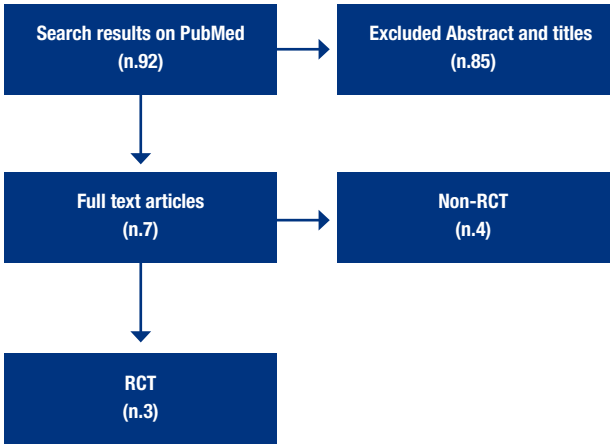
Angina Pectoris, PTCA (Percutaneous Transluminal Coronary Angioplasty), OMT (Optimal Medical Therapy), PCI (Percutaneous Coronary intervention), ACS (Acute Coronary Syndrome), CABG (coronary artery bypass graft), RITA_(Randomized Intervention Treatment of Angina), COURAGE (Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation), JSAP (Japanese Stable Angina Pectoris).

Introduction

Stable angina pectoris is the most common presenting symptom of coronary artery disease (CAD). Epidemiological studies have estimated that 4.6% of US adults over 20 years of age suffer from angina, with as many as 500 000 new cases each year for US adults over 45 years of age.(4) While the optimal management for stable coronary artery disease remains controversial, there are three major options: anti-anginal medication, coronary artery bypass surgery (CABG) and percutaneous coronary intervention (PCI). The use of percutaneous coronary intervention has become common in the management strategy for patients with stable coronary artery disease. Nevertheless, according to treatment guidelines, the combination of intensive medical therapy, lifestyle intervention and reduced of risk factors is the optimal medical therapy. (2) According to recent data, approximately 1,256, 000 coronary stent procedures were performed in 2005 in the United States(4); approximately 85% of all these PCI procedures were undertaken electively in patients with stable chronic artery disease. (1) PCI is very effective in reducing mortality and non-fatal myocardial infarction in patients who present with acute coronary syndromes, but similar benefit has not been shown in patients with coronary artery disease. This topic has been addressed in fewer than 3,000 patients, many of whom were treated before the widespread use of intracoronary stents and current standards of medical management.(2)

Previous studies have shown only that PCI decreases the frequency of angina and improves short-term exercise performance, but the long-term prognostic effect of PCI on cardiovascular events in patients with stable coronary artery disease remains uncertain.(1)

The aim of this systematic review is to review the effectiveness of percutaneous coronary intervention in stable coronary artery disease and to formulate recommendations for the patients with stable coronary artery disease. In order to do this, we systematically reviewed all studies concerning the use of PCI with or without optimal medical therapy in patients with stable coronary disease.



Methods

We systemically searched PubMed(http://www.ncbi.nlm.nih.gov/pubmed). (We started looking for suitable MeSH Database of PubMed).

We searched (on 17th of January 2010, 21-22PM) using the following terms: "Percutaneous Coronary Intervention"[MeSH Major Topic] AND (stable [All Fields] AND ("angina pectoris"[MeSH Terms] OR ("angina"[All Fields] AND "pectoris"[All Fields]) OR "angina pectoris"[All Fields] OR "angina"[All Fields])) AND (Randomized Controlled Trial[ptyp] AND English[lang])

We then made a first selection of articles by looking at the titles and abstracts. We found 92 titles which described stable coronary heart disease and PCI. Of the 92 titles, 85 were excluded because they contained reviews, editorials, letters, practice guidelines and meta-analyses. We did not make a selection based on age or race of subjects.

Data extracted from each study included the first author's name, year of publication. All the patients in our selected groups received aspirin, and those who were undergoing PCI also received clopidogrel in accordance with treatment guidelines. Optimal medical therapy included beta-adrenoceptor blocker, with a calcium antagonist and/or longacting nitrate in maximally tolerated doses (1) Preventive therapy included long-acting metoprolol, amlodipine, and isosorbide mononitrate, alone or in combination, together with simvastatin and either lisinopril or losartan for secondary prevention. Patients were followed for a minimum of 30 months. (2, 3 and 4)

Results

Table-1 Study characteristics

Study	Patients	Follow-up	Region	Date
		median		
COURAGE	2287	4.6 yr	U.S./Canada	April, 2007
RITA-2	1018	2.7 yr	United Kingdom	August, 1997
Nishigaki et al.	384	3.3 yr	Japan	August, 2007

Patients' characteristics

We found no significant differences between the PCI and medical therapy groups in the Nishigaki trial. (3) Data provided by the COURAGE trial showed a significantly higher percentage of proximal left anterior descending coronary artery or LAD disease (P=0.01), but otherwise no significant differences.(4) Treatment groups in the randomized RITA trial-2 were as matter of course also comparable.(1)

Table- 2 All patients' baseline characteristics at randomization (PCI+PCI with OMT)

	COURAGE (n= 2287)	JSAP (n=394)	RITA-2 (n=1018)
Characteristics			
Age, yrs (mean)	61,7 (p=0.54)	64.4 (p=0.755)	58%
Male	85%	75.2% (p=0.930)	82%
Diabetes	33.5% (p=0.12)	40.0% (p=0.900)	9%
Hypertension	66.5% (p=0.53)	63.4% (p=0.992)	-
Congestive heart failure	4.5% (p=0.59)	-	-
Prior myocardial infarction	38.5%(p=0.80)	14.6% (p=0.768)	47%
Previous PCI	15.5%(p=0.49)	26.8% (p=0.337)	
CABG	11% (p=0.94)	2.2% (p= 0.441)	-
Vessels with disease	P=0.72	P=0.998	-
1	30.5%	67.5%	-
2	39%	32.5%	-
3	30.5%	Excluded	-
Medication			
Statin	87.5%	47.2% (p=0.138)	13%
ACE-inhibitor	59%	18% (p=0.068)	10%
Calcium-antagonist	41.5%	57.8% (p=0.145)	50%
Aspirin	95.5%	91.6% (p=0.197)	87%
ß-blocker	87%	47.6% (p=0.129)	67%
Nitrate	67%	54.2%	44%

In the COURAGE study, which included the most participants relative to the other two studies, 1149 patients were assigned to undergo PCI with optimal medical therapy and 1138 were assigned to receive optimal medical therapy alone. (4) The primary outcomes were death from any cause and non-fatal myocard infarction during a follow up of 4.6 years. In the RITA-2 study 504 patients were randomly assigned to the PCI group and 514 to the medical therapy group. The primary endpoints in these studies were the combined frequency of death from all causes and definite non-fatal myocardial infarction. (1) Furthermore, in the JSAP trial (384), 192 patients were assigned to PCI+MT and 192 patients to optimal medical therapy MT. The follow up varied from 2.5 to 7 years. (3)

Primary outcome

The primary outcomes of these randomized controlled trials were death from any cause and myocardial infarction. There were no significant differences between the PCI and medical therapy groups for the COURAGE and Nishigaki trials (unadjusted hazard ratio for the PCI group, 1.05; 95% CI, 0.87 to 1.27 for the COURAGE trial, hazard ratio for the PCI group, 0.87; 95% CI, 0.278 to 2.604 for the Nishigaki trial). (3 and 4) The RITA-2 trial showed a significantly higher risk for the PCI group (relative risk for the PCI group, 1.92; 95% CI 1.08 to 3.41). (1)

Figure 1 - Kaplan-Meier Survival Curves. COURAGE Study
In Panel A, the estimated 4.6-years rate of the composite primary outcome of death from any cause and nonfatal myocardial infarction was 19.09% in the PCI group and 18.5% in the medical therapy group. In Panel B, the estimated 4.6-years rate of death from any cause was 7.6% in the PCI group and 8.3% in the medical therapy group. In Panel C, the estimated 4.6-years rate of hospitalization for acute coronary syndrome(ACS) was 12.4% in the PCI group and 11.8% in the medical therapy group. In Panel D, the estimated 4.6-years rate of acute myocardial infarction was 13.2% in the PCI group and 12.3% in the medical-therapy group.

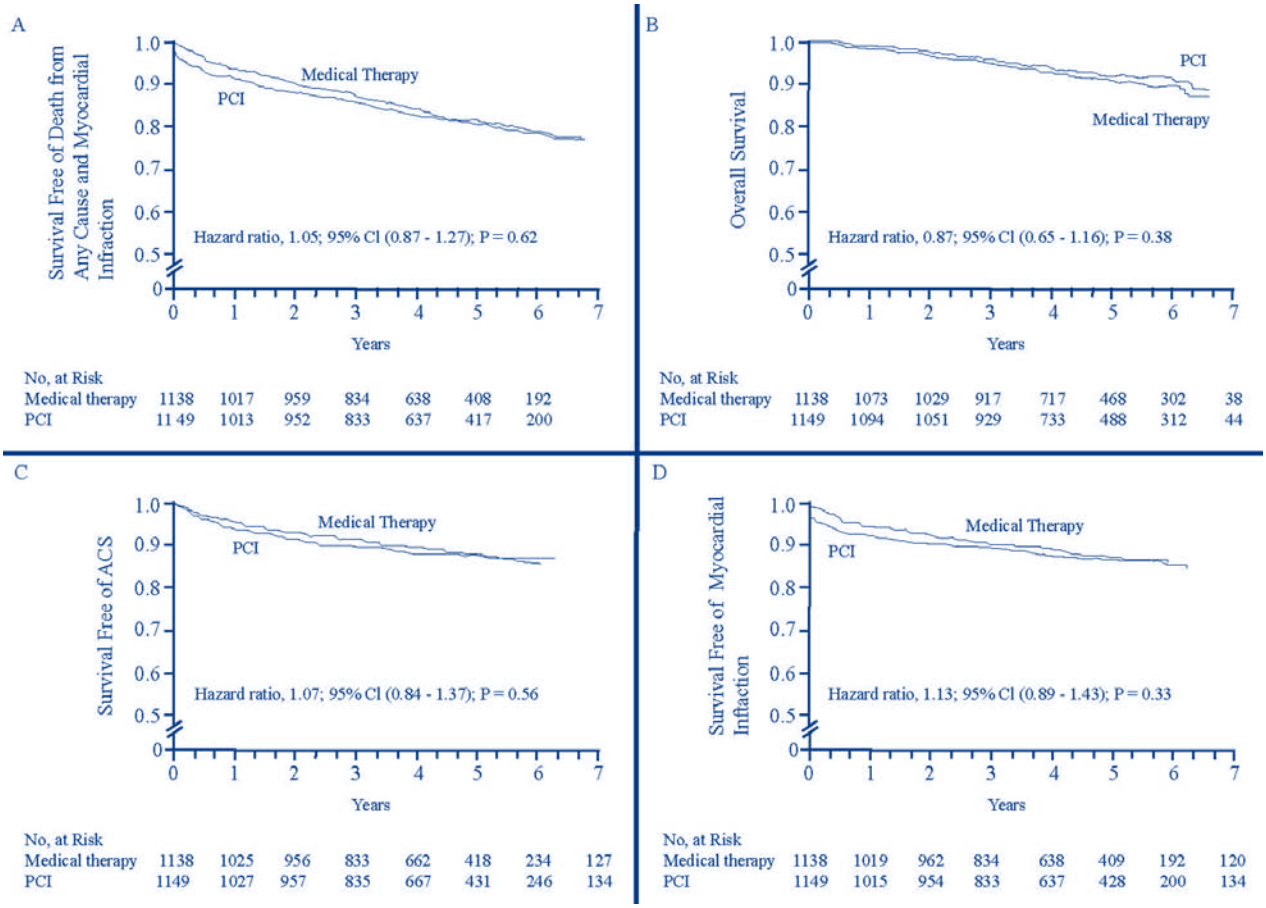
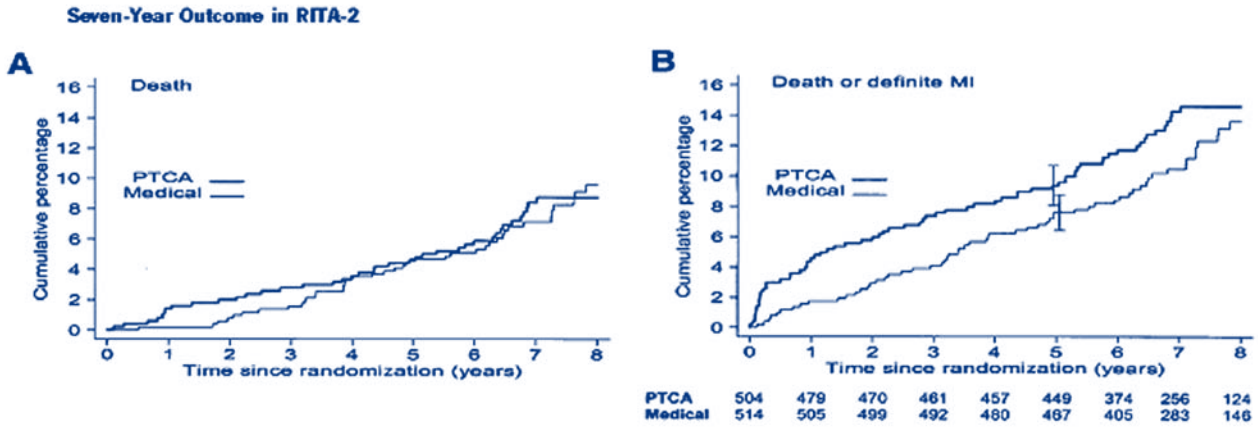


Figure 2 - Cumulative risk of (A) death and (B) death or definite non-fatal myocardial infarction (MI). Bars show 95% confidence interval. Numbers show patients at risk of death or non-fatal MI. RITA-2 Study



Secondary outcomes

We defined secondary outcomes as the occurrence of acute coronary syndrome (ACS), change in class of angina and severity of symptoms, emergency hospitalization, elective and emergency revascularization and stroke. (1, 3 and 4)

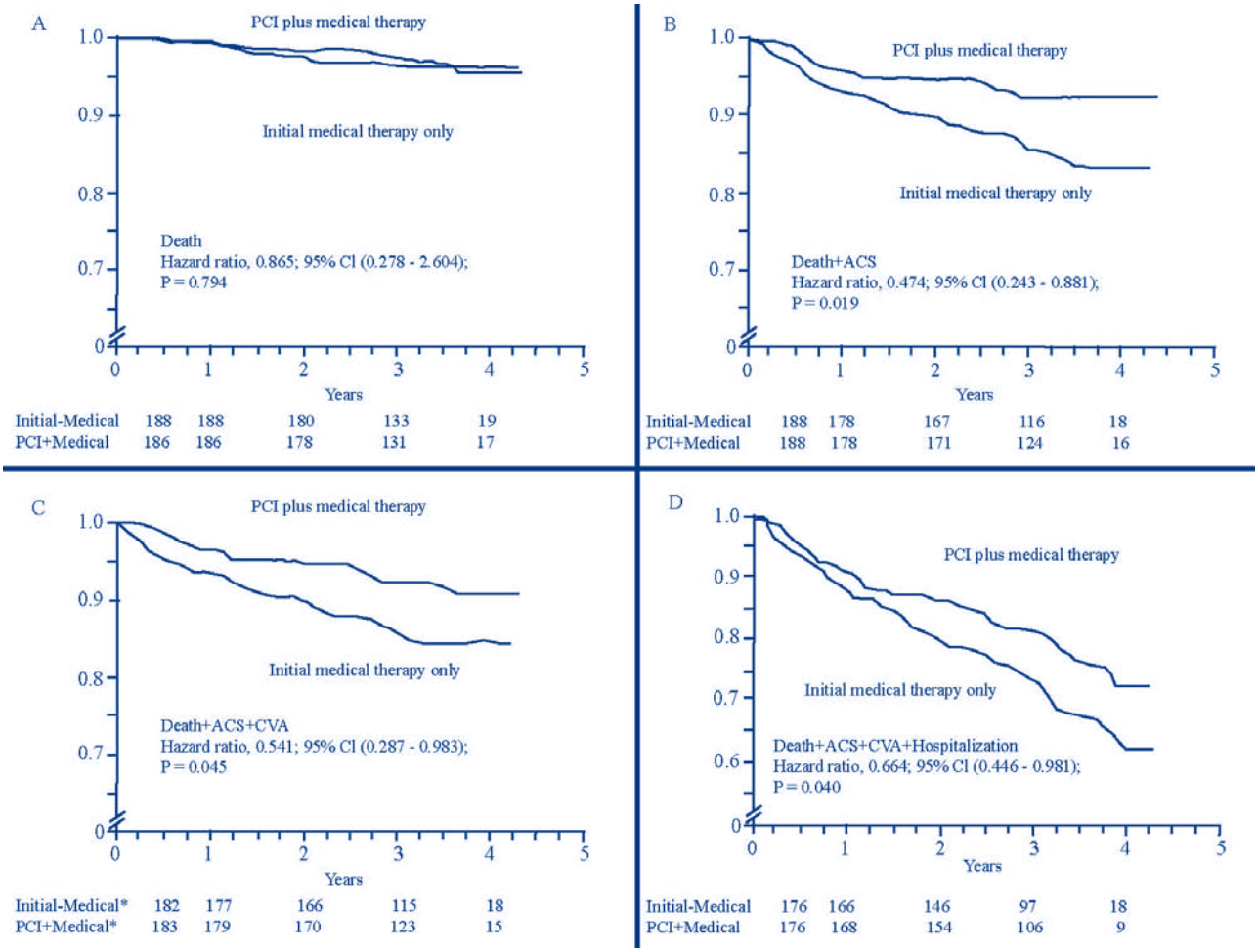
While the COURAGE trial did not find a significant difference in hospitalization for ACS (12.4% and 11.8% for the PCI and medical therapy groups respectively, hazard ratio, 1.13; 95% CI, 0.84 to 1.37) the Nishigaki trial did find dissimilar outcomes (hazard ratio for PCI group, 0.38; 95% CI 0.168 to 0.802). (3 and 4) The RITA-2 trial did not include any numerical data on ACS. However, the authors did report a comparable percentage of patients with a pattern of unstable angina pectoris (9.5% for both groups). (1)

Table 3 - Effect of PCI + medical therapy vs. medical therapy alone in COURAGE, JSAP and RITA-2.

	COURAGE (n=1018)	JSAP (n=394)	RITA-2
	Hazard Ratio (95%CI)	*Hazard Ratio (95%CI)*	*Hazard Ratio (95% CI)*
Death	1.05 (0.65-1.16)	0.87 (0.278-2.604)	1.57 (N/A)
Death and ACS	-	0.47 (0.243-0.881)	-
Death, ACS, and stroke	-	0.54 (0.287-0.983)	-
Death, ACS, stroke, emergency hospitalization	-	0.66 (0.446-0.981)	-
Death, myocardial infarction and stroke	1.05 (0.87-1.27)	-	1.92 (1.08-3.41)
ACS	-	0.38 (0.168-0.802)	-
Myocardial infarction	1.13 (0.89-1.43)	0.43 (0.092-1.542)	-
Unstable angina	-	0.37 (0.133-0.899)	-
Emergency hospitalization	1.07 (0.84-1.37)	0.66 (0.435-0.983)	-

Figure 3 - Kaplan-Meier Survival Curves for the Primary End Point. JSAP Study

Note that the survival curves of all causes of death(Death) + acute coronary syndrome (ACS), Death + ACS + cerebrovascular accidents (CVA) and death + ACS + CVA + Hospitalization (emergency hospitalization) in the PCI plus medical therapy group were shifted significantly upward to that in the optimal medical therapy only group, although Death was similar in the two groups. *The number of patients in each of the PCI plus medical therapy and optimal medical therapy only groups.



All trials showed more improvement which was statistically significant for angina in the PCI groups, although definitions and outcomes were different per trial. (1,3 and 4) The COURAGE trial defined rates of freedom from angina throughout most of the follow-up period; these rates, were significantly higher for the PCI group. However at 5 years, 74% of patients in the PCI group and 72% of those in the medical therapy group were free of angina, which was not significantly different (P=0.35). (2 and 4) Nishigaki et al. reported significantly lower severity of angina symptoms in the PCI group than the group with only medical therapy. (3) The RITA-2 trial described substantial improvement of reported angina in both groups, but relief from angina was better in the PCI group. (1) Also, the difference of grade 2+ angina between groups decreased from 16.5% excess in the medical therapy group 3 months after randomization (p<0.001) to a 7.6% excess of grade 2+ angina (p=0.02). (1, 3 and 4) Nishigaki et al. reported that the rate of emergency hospitalization was significantly lower in the PCI group (20.6%) than in the medical therapy group (31.6%) (P=0.04). (3) Although no data was available for this subject in the RITA-2 and COURAGE trials, hospitalization for ACS in the COURAGE trial was addressed, which we mentioned earlier. (1 and 4)

In the COURAGE trial, revascularization was performed for angina that was unresponsive to maximal medical therapy or when there was objective evidence of worsening ischemia from noninvasive testing, at the discretion of the patient's physician. (4) No data was available for elective or emergency revascularizations separately, but only for total revascularization. At a median follow-up of 4.6 years a significant difference was found, since 21.1% of patients in the PCI group had additional revascularization, compared to 32.6% in the medical therapy group (hazard ratio, 0.60; 95% CI, 0.51 to 0.71). (4) The median time _to _revascularization was 10.0 months for the PCI group and 10.8 months in the medical therapy group. Nishigaki et al. reported elective and emergency revascularization separately. Elective repeat revascularization was performed on a significantly smaller number of patients in the PCI group (21.4%), compared to 36.5% in the medical therapy group (P=0.0011). The same tendency was observed in emergency revascularizations, since 5% of patients in the PCI group needed emergency revascularization for ACS, compared to 12% in the medical therapy group. (3) In the RITA-2 trial 20.2% of the patients in the PCI group required additional revascularization compared to 25.5% in the medical therapy group, but no statistical analysis was performed. (1)

Regarding stroke, there were no significant differences between the PCI group and medical therapy group for any of the three trials, but, again no statistical analysis was available for the RITA-2 trial. (1) The COURAGE trial reported a non significant difference of 22 cases in the PCI group compared to 14 in the medical therapy group (P=0.19). (4) The RITA-2 trial reported 1 case in the PCI group compared to 6 in the medical therapy group. (1) Nishigaki et al., reported 2 cases in each group, which was not a significant difference (P=0.978). (3)

Subgroup analysis

The COURAGE trial was the only trial to conduct a statistical subgroup analysis. According to the outcome, there was no significant interaction (P<0.01) between treatment effect and any predefined subgroup variable. For patients with diabetes, multivessel coronary artery disease and previous myocardial infarction, the primary endpoint was similar in both groups(4)

Discussion

For our systematic review we selected randomized controlled trials (RCT's), but we found only 3 such trials on treatment effect of the medical therapy with or without PCI in patients with stable coronary disease.

Many studies in Western countries and in Japan, some of which were included in this review, found that long-term prognoses with respect to death or acute coronary syndrome were generally not significantly better with PCI+MT than with OMT in patients with CAD. (1,2,3 and 4) In the present systematic review, however, we tried to look at the primary and secondary cardial end-points in patients using MT with or without PCI.

According to the primary results of the COURAGE trial involving patients with stable coronary disease, when PCI was added to optimal therapy as an initial management, there was no significant reduction in long-term rate of composite endpoint, major cardiovascular events or non-fatal myocard infarction.

The RITA-2 trial showed that - compared to medical therapy alone - PCI improved symptoms, reduced the need for anti-anginal medication, and improved exercise tolerance. However, this concerned only short-term differences between the MT group and MT+PCI group. The authors of the RITA-2 trial concluded that the long-term effectiveness of such a policy are unknown. Due to the procedure-related risk of PCI among patients with stable coronary disease, the RITA-2 trial suggested optimal treatment strategies for an important group of patients with angina.

On the other hand, the primary results of the JSAP trial showed that in patients with stable coronary disease and inducible ischemia who were treated with medical therapy, the addition of PCI may improve long-term prognoses more effectively than optimal medical therapy. We think that the procedure-related risk of PCI among patients with stable coronary disease in the RITA-2 trial has consequences for our study, because it was conducted 10 years after the COURAGE and JSAP studies.

There were also significant technical –differences regarding PCI in the JSAP and COURAGE trials. In Japan, PCI is generally performed using intravascular ultrasound. This was true for all of the patients in the PCI+MT group in the JSAP trial, but it is not always true in USA or elsewhere. The initial success rate per vessel for PCI was higher in the JSAP trial (99.5%) than in the COURAGE trial (93%). Perhaps the better initial success rate in the JSAP trial reflects differences in the technical aspects of the PCI procedure.

We found some differences between optimal medical therapy (in the COURAGE trial) and medical therapy alone (in the RITA-2 and JSAP trials).Optimal medical therapy differs from medical therapy in terms of the reduction of risk factors and lifestyle intervention. In the RITA-2 and JSAP trials, patients were assigned to groups with PCI and MT or medical therapy alone. But in the COURAGE trial, patients reduced their risk factors for ACS or any other factors that could lead to endpoint. Due to differences, we believe the results of the COURAGE study are not really comparable with the RITA-2 and JSAP studies.

For the patients in the JSAP trial, who had 1- or 2-vessel disease, the cumulative rate of ACS during the 3.3 year follow-up was 5.0% in the PCI+MT group and 11.7% in the OMT group. In the RITA-2 trial, in which most of the patients had 1-or 2 vessel disease, during the 2.7 years follow-up the cumulative incidence rate of ACS in the PCI+MT group was 13.6% and in the OMT groups it was 11.4%. In the COURAGE trial, in which approximately one-third of the patients had 3-vessel disease, during 4.6-years follow-up the cumulative incidence rates of ACS were 20.0% and 18.7%, respectively.

In the RITA-2 trial, the increase in ACS during the follow-up could be explained by technical problems with the stents, which were used only occasionally at that time but had become popular at the time of JSAP trial. Furthermore, patients with 3-vessel disease were included in the COURAGE trial (33%), and it is now known that 3-vessel disease is the most important risk factor for cardiac events after PCI. (2) All of the subjects in JSAP were stable CAD patients with 1-or 2 vessel disease. The background of the patients and the fact that the JSAP study used the PCI procedure more frequently, could therefore help to explain differences between the RITA-2 trial, the COURAGE trial and the JSAP trial.

The number of participants in the included studies was relatively small, given the large volume of patients undergoing elective PCI. Therefore, we think a large, multicentre randomized controlled trial is necessary to obtain hard evidence of the effect of the medical therapy with or without PCI in patients with stable coronary disease.

Conclusion

Although previous studies have shown the PCI decreases the frequency of angina and improves short-term exercise performance, medical therapy compared with PCI plus medical therapy results in no significant difference in long-term cardiovascular outcome in patients with stable coronary disease. (1, 2, 3 and 4) Due to certain technical aspects of the PCI procedure, an initial strategy of (optimal) medical therapy in patients with stable coronary artery disease currently seems to be the best option. (2)

References

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Combination therapy enhances effect of NB-UVB for treatment of vitiligo.

A systematic review.

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Objective: **To determine the most effective treatment for vitiligo: narrow band (NB)-UVB monotherapy or combination therapy using NB-UVB with various agents.**
Methods: **We searched the PubMed database using the Mesh terms "vitiligo/therapy" and "ultraviolet therapy." We selected randomized controlled trials and only included studies that compared NB-UVB monotherapy with NB-UVB combination therapy.**
Results: **We included 6 articles. Two studies compared NB-UVB monotherapy with combination therapy using antioxidants; both showed significant improvement of repigmentation when an antioxidant was added. Two other studies compared NB-UVB monotherapy with combination therapy using the vitamin D derivative tacalcitol; they also reported significant improvement. The remaining two studies reported no significant improvement. One of these compared NB-UVB monotherapy with combination therapy using tacrolimus (macrolide isolate); the other study compared NB-UVB monotherapy with combination therapy using the immunomodulator polypodium leucotomos.**
Conclusions: **Combining antioxidants or tacalcitol with NB-UVB improves repigmentation. It is unclear which agent is more effective, but due to the more serious side-effects from tacalcitol treatment, we suggest that the combination of NB-UVB with antioxidants is currently the treatment of choice for vitiligo.**

Keywords

Vitiligo/therapy; Ultraviolet therapy

Introduction

Vitiligo is an acquired disorder of the skin, which is characterized by depigmentation. It affects approximately 1-2% of the world population, with no predilection for age and gender. Although vitiligo is not a life threatening disease, it is a disfiguring disorder and can have psychological consequences.(6)

The progressive depigmentation is associated with loss of melanocytes from the basal layer of the epidermis. Although the pathogenesis remains elusive, several theories have been proposed to explain this loss of epidermal melanocytes. These theories, as described in the review of T. Forschner about the current state of vitiligo (4), are listed in Box 1. Studies have also pointed to a significant role for genetic susceptibility in vitiligo, evidenced by familial occurrence in about 30% of patients.(1)

Because of the unknown pathogenesis, many treatment strategies have been devised. Some of these treatments were developed to restore the functional integrity of the epidermis and melanocytes by reactivating residual melanocytes, while others suppressed the immune reaction. Phototherapy aims for both effects; according to the most recent guidelines for the treatment of vitiligo, narrow band ultraviolet B (NB-UVB) is the first choice of treatment in generalized vitiligo. However, many studies have suggested that NB-UVB therapy is more effective in combination with another agent. The agents that have been proposed for combination therapy (Box 2) affect different mechanisms and have shown variable results.

We designed a systematic review to 1) compare the clinical efficacy of NB-UVB monotherapy with therapies that combine one of the four agents with NB-UVB treatment, and 2) to rank the efficacy of these agents when used in combination therapy.

Box 1 - Theories of pathogenesis

1. *Autoimmune theory:* Vitiligo often appears along other autoimmune diseases and patients have auto antibodies towards melanocytic antigens. There is also evidence for T cell involvement.
2. *Neural theory:* Altered reactions of melanocytes towards neuropeptides, catecholamines and their metabolites are responsible for melanocyte destruction.
3. *Self-destructing theory:* Defects in protective mechanisms of the melanocytes, resulting in accumulation of melanotoxic indole derivates and free radicals, cause self-destruction.
4. *Biochemical theory:* An overproduction of hydrobiopterin, a cofactor of tyrosine hydroxylase, leads to increased catecholamine synthesis, causing an increase in melanotoxic reactive oxygen species.

Box 2 - Agents and their function

1. *Antioxidants:* Reduce oxidative stress.
2. *Tacalcitol:* Vitamin D3 analogue that inhibits T-cell activation, stimulates differentiation of keratinocytes and induces melanogenesis by reducing the disturbed Ca2+ influx into melanocytes.
3. *Polypodium Leucotomos:* A type of fern of which the extract has photo-protective and immunomodulatory properties.
4. *Tacrolimus:* : Macrolide that suppresses the expression of proinflammatory cytokines and inhibits T-cell signaling pathways.

Methods

On January 18th 2010 we searched the PubMed data bank. The MESH-terms used were: “Vitiligo/therapy” and “Ultraviolet therapy”. We restricted the search for “Vitiligo/therapy” to major topic headings only and selected randomized controlled trials and articles published in English. Furthermore, we filtered for ‘Erasmus MC free full text’, because we do not have free access to other articles. After reading the title and abstract, we only included articles that compared NB-UVB monotherapy with combination therapy using NB-UVB and an agent.

Results

Our PubMed search yielded 26 publications. After applying the inclusion criterion “comparison of NB-UVB monotherapy with NB-UVB combination therapy” 7 articles remained. Unfortunately we did not have access to an online version of the article written by M. Tjioe et al. about the addition of vitamin B12 and folic acid, neither was this article available in the university library. Therefore 6 articles remained for analysis, all of which compared the effectiveness of NB-UVB as a monotherapy with NB-UVB in combination with different agents. The results of these publications are summarized in Table 2. The study characteristics are summarized in Table 1.

Patients

The number of subjects ranged from 9 to 50. In all studies, except for one (9), the subjects were older than 18 years. All studies excluded patients with segmental vitiligo, patients at risk (hyper photosensitive, history of skin malignancy, pregnant) and patients who had previous phototherapy or another treatment for vitiligo within the last 3 months.

Table 1 - Study characteristics

Study (reference number)	Blinding	Patient number
NB+antioxidants	double	35
NB+antioxidants	none	24
NB+vitamin D	none	32
NB+vitamin D	none	38
NB+tacrolimus	double	9
NB+polypodium	double	50

Treatment

Some studies (1, 6, 7, 9) started their treatment with a 70% Minimal Erythema Dose (MED) of NB-UVB. The remaining studies defined their starting dose in J/cm2 (0.196 and 0.21). The dose was increased for every subsequent treatment. However, when minimal asymptomatic erythema occurred, the dose was kept constant. If symptomatic erythema developed, the dose was decreased. The various treatments took 3 to 6 months. The frequency of NB-UVB therapy varied from 1 to 3 times per week. The frequency of administration of the supplement agent varied from 1 to 3 times per day. The main outcome of all studies was repigmentation. The studies utilized the same classification of repigmentation: “none”, “moderate”, “good” and “excellent”. However, the definitions for each class (percentages of repigmentation) differed somewhat. To evaluate the repigmentation, vitiligo lesions were photographed before treatment and at the end of the study.

Overall, the best responses to therapy were found in the face and neck regions; feet and hands showed the least repigmentation.

Agents

Overall, combination therapy showed more repigmentation than monotherapy with NB-UVB.

Antioxidants
Two studies (7, 8) compared NB-UVB monotherapy with the combination of NB-UVB and antioxidants. In both studies, combination therapy with antioxidants resulted in significantly more repigmentation relative to monotherapy (34% versus 17%). One study (7) reported that adverse effects were minimal.

Tacalcitol (vitamin D)
Two studies compared NB-UVB monotherapy with the combination of NB-UVB and the vitamin D analogue tacalcitol. These studies showed an improvement in the extent of repigmentation with addition of topical tacalcitol. This improvement (50% versus 20%) was statistically significant. The side effects of tacalcitol were limited to mild erythema, xerosis and itching.(1, 9) Mild irritation and desquamation of the face was described in one article.(1) In lesions treated with tacalcitol, the time to repigmentation was significantly shorter.

Polypodium leucotomos(11)
One study compared NB-UVB monotherapy with NB-UVB and the immunomodulator polypodium leucotomos. The difference in effectiveness of monotherapy versus this combination therapy was not significant for all body areas. When only data from patients who participated in more than 80% of the NB-UVB sessions were analyzed, more repigmentation in the head and neck areas was observed in the group treated with polypodium leucotomos. This finding was statistically significant (P<0.002).

Tacrolimus(10)
The remaining study compared NB-UVB monotherapy with NB-UVB and tacrolimus (macrolide isolate). No statistically significant differences were found between tacrolimus-treated lesions and placebo-treated lesions.

Discussion/ Conclusion

Vitiligo treatment encompasses various strategies. However, narrow band UVB therapy is the first choice for treatment of generalized vitiligo disease. Several studies hypothesized that combining NB-UVB monotherapy with an agent could increase the effectiveness in repigmentation. In this systematic review, we summarized the results of these studies.

Conclusions
We found that two combination treatments significantly increased repigmentation in patients with vitiligo: 1) supplementation with an antioxidant; 2) supplementation with the vitamin D analogue tacalcitol. Addition of tacrolimus or polypodium leucotomos did not improve the repigmentation significantly, and therefore were not taken into further consideration.

Because the study designs differed in terms of frequency of NB-UVB treatment, frequency of antioxidant/ tacalcitol administration and treatment duration, it is difficult to conclude which combination treatment is preferable. However, application of topical tacalcitol seemed to cause more side effects (itching, xerosis, erythema and desquamation in the face). Therefore, based on current information, we conclude that combining NB-UVB with antioxidants is the best treatment for vitiligo.

Discussion
Note that these conclusions are based on only 6 articles and that none of these studies included more than 50 subjects. Therefore our conclusions have a narrow evidence base.

Another point of discussion is that of all the studies that described the improvement as significant (P<0.05), none reported the exact P-value; statistical analysis was lacking.

There was a large difference in the repigmentation improvements found by the two studies that examined combination therapy with antioxidants.(7, 8) This can probably be attributed to the fact that in the study describing a 3.6-fold increase of repigmentation, the subjects took an antioxidant capsule twice daily, compared to once daily in the study that described a 1.3-fold improvement. However in the group where a 3.6-fold improvement was found, subjects received NB-UVB therapy only 2 times per week, compared to 3 times per week in the other study. This suggests that antioxidants may contribute more to repigmentation than NB-UVB does.

A similar difference was found in the studies that investigated tacalcitol ointment.(1, 9) However in this case the relative contribution of NB-UVB and tacalcitol seems to be reversed, with NB-UVB being more important for the repigmentation. This difference could be attributed to the fact that the study with the biggest improvement utilized a cut-off at 80% to describe "excellent" repigmentation, compared to a 75% cut-off used in the other studies. Due to this high cut-off, no patients belonged to the "excellent" group when treated only with NB-UVB.

In G. Leone et al. (1), the researchers found that response to treatment in both groups was related to the duration of the disease; patients with shorter disease duration had a better response. This suggests that therapy should be started as early as possible.

This review indicates that improved repigmentation can result from combination treatment using NB-UVB with antioxidants or tacalcitol. However, more research must be done so this conclusion can be based on adequate evidence. Another focus for subsequent research could be combination therapy with another vitamin D analogue which has possibly fewer side effects. A third possibility is "triple" combination therapy with NB-UVB, antioxidants and tacalcitol.

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Table 2 - Overview of studies

Study (reference number)	Repigmentation (%)	Patients with repigmentation NB-UVB (%)	Patients with repigmentation NB-UVB + an agent (%)	Frequency NB-UVB treatment, treatment duration	Frequency of agent administration (/day)	Compliance (%)
	>75	13	47	2x /week,	2 (capsule,	80
NB-UVB + placebo				6 months	started 8 weeks	
vs.					before NB-UVB)	
NB-UVB + antioxidants						
	>75	56	73	3x /week,	1 (capsule,	83
NB-UVB				6 months	started 2 weeks	
vs.					before NB-UVB)	
NB-UVB + antioxidants						
	>80	0	50	2x /week,	1 (ointment)	100
NB-UVB				6 months		
vs.						
NB-UVB + vitamin D						
	>75	6	26	1x /week,	2 (ointment)	92
NB-UVB				3 months		
vs.						
NB-UVB + vitamin D						
	No statistically significant			3x /week,	1 (ointment)	89
NB-UVB + placebo	differences			3 months		
vs.						
NB-UVB + tacrolimus						
	No statistically significant			2x /week,	3 (capsule)	98
NB-UVB + placebo	differences			6 months		
vs.						
NB-UVB + polypodium leucotomos						

Should we record the DNA profiles of the entire Dutch population in a DNA database?

An opinion.

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Summary: **There are many ways to identify a person who has left a DNA trace at a crime scene, of which the DNA database probably is the most frequently discussed. Such a database already exists, but the number of DNA profiles recorded is small.**

In this article, we answer the question of whether we should set up a DNA database containing the DNA profiles of the entire Dutch population to aid forensic DNA research. To answer this question, we determined the ideal conditions for the functioning of this database and which of these conditions can be satisfied. In this way, we found the bottlenecks for introducing this population-wide DNA database.

We conclude that the expected benefits of a population-wide DNA database do not outweigh the bottlenecks, ethical drawbacks and necessary investments.

Introduction

The use of DNA as evidence in court is booming. Many prisoners have been released because DNA research has proven their innocence. On the other hand, DNA research has limitations; in many cases DNA traces have been found, but cannot be linked to the person who has left the trace behind. What are the possibilities for matching a DNA trace to a suspect?

Firstly, one could perform a "dragnet investigation", in which hundreds to thousands of people in the vicinity of the crime scene voluntarily submit to DNA testing. The retrieved DNA is compared with DNA found at the crime scene. It is generally assumed that criminals do not cooperate with DNA testing, though. And there are other reasons not to cooperate.

Secondly, researchers are investigating the possibility of predicting externally visible characteristics of the person who left the DNA trace, using only the DNA trace. However, many visible characteristics, like the size of the forehead, have a complex genetic basis. These characteristics cannot yet be predicted using DNA alone.

A third method to match a DNA trace to a suspect is to use a DNA database that contains the DNA profiles of all inhabitants of a country. This database allows matching any DNA trace to its owner. Because the DNA donation is universal, this method does not have the disadvantage of "dragnet investigation" (i.e. voluntary donation). Moreover, it does not have the complexity of predicting externally visible characteristics.

In this article, we answer the question of whether we should record the DNA profiles of the entire Dutch population in a DNA database to aid forensic research. For this purpose, we first describe the current situation of DNA databases. Next, we discuss the implications of setting up a DNA database that contains the DNA profiles of all Dutch inhabitants. We conclude by addressing the question whether this database should actually be introduced.

The current situation regarding DNA databases

Technical background

To save DNA in an electronic database, you can theoretically determine the sequence of the complete DNA and save this information. However, this presently costs hundreds of thousands of euros per person, although the costs are expected to decrease as time goes on (1).In practice, non-coding DNA is used. This is DNA that does not code for proteins or other products. This DNA contains hypervariable sequences, which are called short tandem repeats (STRs) (2). These STRs are repeats of sequences of two to ten bases, for example (CA)n, in which n is the number of repeats in the sequence, for example 12. This number (the length of an STR) varies from person to person. By determining the length of a larger number of STRs, the chance of finding a match of the DNA profiles of genetically different persons is minimized. Such a DNA profile essentially consists of only a few numbers.

Modern DNA databases

In the Netherlands, an individual's DNA profile is saved when he or she is suspect of or tried for a crime for which the law allows a maximum imprisonment of 4 years or more. Moreover, the result of the DNA analysis must be 'necessary for the investigation' (3). A suspect's DNA is not saved when he/she confesses to the crime, and it is deleted from the Dutch DNA database after a certain period of time. The exact duration of storage before deletion depends on the duration of imprisonment as imposed by the court: 20 years storage for imprisonment of four to six years and 30 years storage for imprisonment of more than six years (4). DNA profiles are also deleted from the database when a suspect is found to be innocent, or when the case is dismissed. The aim of recording DNA profiles in accordance with the conditions mentioned above is to create a database with the DNA profiles of as many members of the criminal population as possible. This is logical, since less than 1% of the entire population is responsible for all violent and sex offences. (5)

The Dutch DNA database now contains approximately 90,000 DNA profiles of individuals (6). DNA profiles of diseased victims and of DNA traces taken from a crime scene are also recorded. With such a database, it is possible to match new crimes to known criminals by comparing DNA traces found at a crime scene with DNA profiles in the database. In the Netherlands, this yields

80 matches per week, mostly consisting of "mass criminality" (89.8%), such as burglaries and vehicle thefts. (7)

If attempts to match DNA traces to individuals fail, then it is possible to compare the profile of a DNA trace with the profiles of traces that have already been recorded in the database, facilitating the linking of crimes. It is also possible to compare DNA profiles of traces with profiles in foreign databases thanks to international treaties. This results in about one match per day.

A major disadvantage of the Dutch database is that it does not contain DNA profiles of people who never got into trouble with the police. Consequently, DNA traces of first-time offenders cannot be linked to individuals by using the DNA database. However, this would be possible with a population-wide database.

A DNA database of the entire Dutch population

Recently, the United Arab Emirates decided to set up a DNA database of their entire population. In the next ten years they intend to make one million profiles per year (8). Should this also be done in the Netherlands?

In this section we discuss which circumstances are ideal for the functioning of a DNA database of the entire Dutch population, and which of these circumstances are realistic. This automatically shows us the bottlenecks of a national DNA database.

In our view, the ideal conditions for the functioning of a population-wide DNA database are the following:

1. Every criminal leaves his or her DNA at a crime scene,
2. This DNA is always found,
3. The DNA is good quality,
4. The criminal's DNA is the only DNA found at the crime scene.
5. The DNA profiles of all inhabitants and visitors of the country have been recorded in the national DNA database,
6. These profiles are error-free,
7. Each profile is absolutely unique,
8. The institution that manages the database has sufficient capacity and guarantees the security of the database.

Below we discuss to what extent we can achieve the ideal circumstances and what problems occur.

1: Not all criminals leave their DNA at a crime scene

Not every criminal leaves behind sufficient DNA to make a DNA profile, even though very small amounts are required. Even while reading this journal you lose skin cells, which contain enough DNA to make a profile (9). However, in many cases criminals do not leave DNA behind, for example when they shoot someone from a distance. Helen Wallace, director of GeneWatch, estimates that a DNA profile can be made at less than 1% of the total number of crime scenes. (10)

2: DNA that criminals leave behind at a crime scene is not always found

Of course, what you find depends on how well you look. This also holds for finding DNA left at a crime scene. In practice, DNA is sampled only if the result is important for police investigations. In addition, it is impossible to make a DNA profile if all DNA has been destroyed, for example in case of arson.

3: DNA that criminals leave behind at a crime scene is not always good quality

Wet surroundings are one of the largest enemies of DNA (11). When DNA has been damaged, it might be impossible to determine the length of some short tandem repeats. Then the DNA profile is not complete and cannot be compared to profiles in the DNA database. Sometimes the damage can be repaired and analysis can still take place. However, the capabilities of this repairing technique are limited.

4: The criminal's DNA is not always the only DNA found at the crime scene

Wherever people are, DNA is left behind. Thus, DNA samples are often mixtures of DNA of the victim, the criminal and people who are not involved in the crime. It is even possible to transmit DNA from person A to person C via person B or via an object. This is called secondary DNA transfer. However, American investigators have concluded that secondary DNA transfer does not compromise DNA research. (12)

5: The national database cannot contain the DNA profiles of all inhabitants and visitors of the country

Retrieving DNA from all legal inhabitants of the Netherlands is one challenge. But many people in the Netherlands have no Dutch passport (whether they are in the country legally or illegally), and these people can also commit a crime. In practice, it is impossible to obtain DNA from those people. (13)

6: No mistakes are made in making and storing DNA profiles

Small quantities of sampled DNA might lead to errors in profiling. Nowadays, advanced techniques limit the risk of mistakes. (14)

7: Each profile is unique

A limited number of STRs is used to make a DNA profile, and each of these STRs has a limited number of possible lengths. Consequently, the number of DNA profiles is limited. According to European guidelines, to minimize the possibility of a false-positive match, seven DNA markers should match.

8: The institution that manages the database has sufficient capacity and guarantees the security of the database

The privacy of people who have their DNA profile stored in the DNA database is guaranteed by the fact that a profile only contains numbers, and therefore cannot be linked directly to a name. The accompanying name can be retrieved only if there is a match (15). The database can only be accessed via a secure connection, and access is only given to a small group of people.

Ethical questions about national DNA databases

Introducing a DNA database for the entire population is accompanied by ethical issues. Specifically, Articles 10 and 11 of the Dutch Constitution (16) apply to these issues. Article 10 describes the right to protection of privacy. Opponents of DNA databases believe that genetic information will be insufficiently protected if there is mandatory donation of DNA. Article 11 describes the right to physical integrity. Opponents say that the donation of DNA is a violation of bodily autonomy. Furthermore, they are afraid that judges will consider DNA as indisputable evidence and that someone who refuses to donate DNA will be seen as a suspect. (17)

Should DNA profiles from the entire Dutch population be recorded in a DNA database?

It is clear that no agreement has been reached on the introduction of a DNA database of the entire Dutch population. Although a database could help to solve crimes, is a DNA database advisable? Our answer to this question is based on an analysis of the feasibility and the effectiveness of such a DNA database.

Setting up a DNA database (feasibility)
First of all, due to practical limitations, DNA profiling is performed on only a small proportion of the crime scenes since DNA is not always found at a crime scene. Moreover, a DNA profile cannot always be made because DNA is subject to degenerative processes and is therefore not always of sufficient quality and quantity. Secondly, there are ethical drawbacks, such as the possible violation of privacy and integrity. Thirdly, it is impossible to obtain DNA from all foreign people in the Netherlands. Fourthly, the costs for setting up and maintaining a database are high. According to Dr. Van Beek, head of the Dutch DNA database department of Netherlands Forensic Institute, it will cost around €1.65 billion to set up a DNA database with profiles of the current Dutch population. With current levels of equipment and staff, this process would take approximately 330 years. This could be accelerated by buying more equipment and hiring more employees, but these investments must be weighed against the expected results.

Using a DNA database of the entire population (effectiveness)
A DNA database of the entire Dutch population would make it possible to link any DNA sample to its owner, if there were no factors limiting the effectiveness of a DNA database.

Firstly, DNA samples could be a mixture of the DNA from several people. Moreover, forgery of evidence is possible. Consequently, a DNA match is not proof of guilt. Furthermore, less than 1% of the Dutch population is responsible for all offences committed in the Netherlands. All other people are innocent and will remain innocent. This means that retrieval of DNA from those people is useless for solving crimes.

Conclusion

We conclude that the expected benefits of a population-wide DNA database do not outweigh the bottlenecks, ethical drawbacks and the necessary investments. The aim of a DNA database is to store DNA profiles of people who could commit a crime in the future. By setting up a population-wide DNA database, these people cannot be missed. On the other hand, 99% of the profiles would be recorded for no purpose. It is perhaps more realistic to obtain DNA in cases of a less serious crime or suspicion of such a crime. Although the DNA database would contain a smaller percentage of the abovementioned target group, it would contain many fewer profiles of people who will never commit a crime. This database would have fewer disadvantages than a population-wide DNA database. The results could be improved by extending international cooperation.

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'Crime Passionel: Emotional rage or cold-blooded murder?'

A review of emotion-induced dissociation in healthy individuals.

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Abstract

Dissociation is a disruption in the usually integrated functions of consciousness, memory, identity or perception of the environment (1). It is mostly seen in the form of dissociative disorders, but it can also occur in healthy individuals (2). When a normal individual claims that a crime was committed in a dissociative state, this is called the non-mental disorder automatism defence (3). If successful, this defence can lead to full acquittal (4). In this paper the processes of dissociation, emotion regulation and the role of emotions on decision making are discussed. It is demonstrated that these three processes share many similarities in development and in activation of neurophysiological structures. The aim of this review is to understand de mechanisms behind decision-making in an emotion-induced dissociative state. Why do people commit impulsive and violent acts in this state, which they would not have committed if they were not dissociated? Finally, a theory that might explain this phenomenon is proposed.

Keyword - dissociation, automatism, emotions, decision making, somatic marker theory

Introduction

Imagine yourself, a healthy individual without any form of mental disorder, coming home unexpectedly a little earlier from work. When you silently enter the house to surprise your wife, you catch her and your best friend in bed together.

A jealous rage occurs and a few hours later the only thing you can tell the investigator is that it felt like a fuse blew up in your head. Everything went black before your eyes and the next thing you can remember is sitting next to two dead bodies with a kitchen knife in your hand.

You have committed a murder, but without any awareness or memory of the act. This is known as dissociation. If this is the case, should you be convicted for your crime? If murder is committed in a state of dissociation, this can be used as a legal defence (2). This mostly seen in non-premeditated intimate partner homicide, popularly referred to as Crime Passionel. A legal defence based on dissociation depends on a psychiatrist's opinion about whether or not it was likely that the accused was in a dissociative state while committing the crime.

In the Netherlands, judges decide in accordance with the psychiatrist's opinion in almost 95% of the cases (5). For the accused, a successful defence based on dissociation can result in full acquittal. However, committing a crime in a dissociative state without any previous history of mental problems is extremely rare. The offender might not always have a criminal record, but usually has a history of mental health problems, drugs and alcohol abuse and/or trauma in childhood (2,4,6)

Of the legal cases examined in this study, almost all that concerned dissociative murder committed by an individual without an underlying mental disorder indicated that dissociation occurred during an argument or a fight (2,3,4). Apparently, a dissociative state in this scenario is most likely preceded by a highly emotional state (5).

The aim of this review is to understand the mechanisms behind decision-making in an emotion-induced dissociative state. To achieve this, understanding is required of three aspects: decision-making, emotions and dissociation. The results section starts with dissociation, since an understanding of this term is necessary to understand the context in which the rest of the review is written. The juridical aspects of dissociation are also discussed. After dissociation, the aspect of emotions is analyzed, since they are a likely cause of dissociation in case of a Crime passionel. The nature and purpose of emotions is also discussed, followed by their role in decision-making and the way emotions are regulated. After examining these aspects and their possible relationships to each other, an integrated theoretical model is proposed that might improve our understanding of why people can act impulsively, violently and even commit murder in a state of emotion-induced dissociation.

Methods

Relatively little has been published about emotion-induced dissociation. Also, the search for relevant information about its different aspects is challenged by the fact that this information is spread over different lines of research: mostly psychiatry, forensics, neuroscience and psychology. Often each of these perspectives required their own search action with specific MeSH terms in order to get information about the same subject. This made it difficult to work with a defined set of inclusion and exclusion criteria.

Since a broad range of subjects related to Crime Passionel were analyzed in this review and data availability was limited, the search strategy consisted out of many small PUBMED searches using different combinations of MeSH terms. This was followed by manual filtering to search for relevant articles. The PUBMED option 'relevant articles' was also used, and references of articles considered relevant for this review were checked for complementary information. Searches were conducted in the period between 07/09/09 and 20/01/10. Only articles registered in the PUBMED/MEDLINE database were used.

Results

Dissociation

Dissociation is defined in the Diagnostic and Statistical Manual of Mental Disorders as "a disruption in the four usually integrated functions of consciousness, memory, identity, or perception of the environment" (1). During a state of dissociation, one or more of these functions can 'dissociate' from the others. This can occur with any of the four functions mentioned above.

Memory function is almost always involved when dealing with dissociation. This leads to dissociative amnesia (4). When other components are involved in combination with dissociative amnesia, this can lead to a dissociative fugue. This is defined as a sudden change in location and identity with complete amnesia for the change (7). When only the perception of the environment is altered, a state of derealisation can occur in which the environment is perceived as unreal or a trusted environment is not recognized (1).

When identity is affected, it is not the environment, but the person's own body that is experienced differently. This is called depersonalization, in which people feel uncomfortably detached from their own senses and surrounding events, as if they were an outside observer (1). People often describe this as being a spectator of their own actions, sometimes even from a third person perspective. It can feel like the body acts automatically without the ability to control it.

Dissociation of consciousness is more difficult to imagine. How could a person function if no consciousness is present at all, would one not just become unconscious? The best way to illustrate that this is also possible, is with the case of Mr. A, who in 1996 murdered his wife by drowning her in the pool. Despite the fact that he obviously committed the crime, he received full acquittal on account of non-mental disorder automatism. His legal defence: sleepwalking (8).

Dissociation can occur in any individual when faced with an overwhelming, traumatic, stressful and/or fearful experience (9,10). In this role dissociation should not be seen as a pathological mechanism, but as a coping mechanism serving to block a painful event from full awareness (4,10). It can be considered adaptive in the sense that it allows a person to carry on with his life as if nothing traumatic has happened (7). However, dissociation is not merely seen as a reaction to stressful events. It can also manifest itself in a pathological way as seen in dissociative disorders such as Dissociative Identity Disorders (DID) and depersonalization disorder (1,11). Dissociation can also arise due to concussion, alcohol intoxication, drugs, lack of sleep and meditation (12).

Causes of dissociation

There is no consensus yet on what exactly causes dissociation (7,9). Dissociative disorders are frequently described in patients with a history of rape and sexual childhood abuse (2), family problems (7), other trauma occurring during their youth (13) and in people suffering from Post Traumatic Stress Disorder (14). This could suggest that frequent use of dissociation as a coping mechanism might eventually predispose an individual to reflexively resort to dissociative behaviour, thereby increasing the chance of developing a dissociative disorder (7).

Another influential theory relating childhood development concerns the development of a region of the prefrontal cortex called the orbitofrontal cortex. If the experience-dependent maturation of this brain region is altered due to trauma exposure, it may be responsible for a pattern of lateral inhibition between conflicting subsets of self-representations which are normally integrated into a unified self (13).

When considering a dissociative state resulting from emotional stress or a traumatic experience, it has also been proposed that Temporal Lobe Epilepsy (TLE) could be responsible (7). It has been demonstrated that emotional stress and trauma can indeed precipitate TLE (7). It has also been observed that epileptic patients share many symptoms with DID patients, including blackouts, fugues, depersonalization and déjà vu (7). Therefore one theory is that the high emotional arousal could cause TLE, which in turn causes dissociation. Criticism of this theory includes the observation that TLE can affect the emotion regulation system in such a way that previous neutral stimuli are experienced with much greater emotion (7). It could be argued then that it is the other way around, and that TLE only intensifies the emotional arousal that leads to a dissociative state instead of causing a dissociative state itself.

Although there is still uncertainty about the cause of dissociation, physiological manifestations during a dissociative state have been subject to various studies (13,15,16). This is possible since various dissociative states can be induced in test subjects both pharmacologically (17) and non-pharmacologically (11).

Two observations found in these types of study are of interest to this paper. Firstly, that while appraisal of the emotional significance of emotional stimuli is intact in depersonalized patients, an affective state is not induced in response to this material (15). Secondly, depersonalized patients display significantly reduced skin conductance in reaction to unpleasant pictures compared to normal individuals, demonstrating a decreased autonomic response (16). Both these findings support the clinical observation that dissociative states which include depersonalization are often accompanied by emotional numbing (15,16).

Dissociation in court

As demonstrated in the introduction and by the brief example of sleepwalking, dissociation can have a major impact in the courtroom. This section explains how a person can receive full acquittal for his crime due to dissociation.

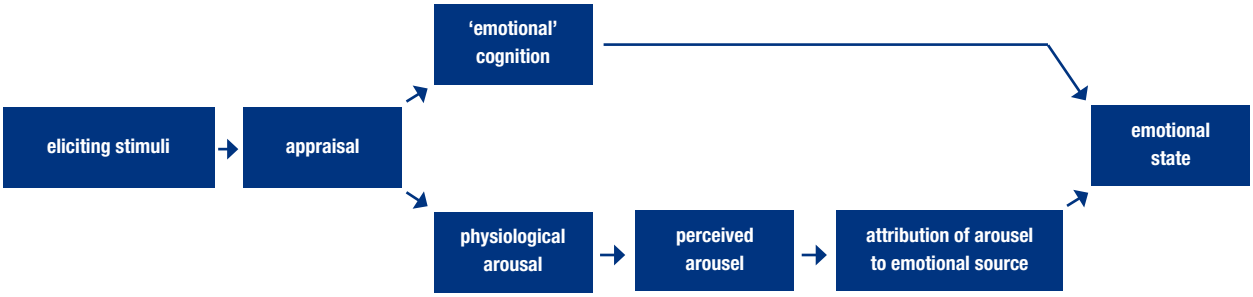
The law requires two components to be present before invidualls can be convicted for their crimes. Firstly there is the Actus reus or "guilty act" and secondly there is the Mens rea or "guilty mind". If the act is present, but is not committed with a guilty mind, a person cannot be convicted for their crime. The main reason for the absence of a guilty mind is an underlying mental disorder. For this reason offenders belonging to the non mens rea category are usually sentenced to treatment at a secure mental hospital instead of imprisonment.

During a crime involving dissociation a guilty mind is usually not present, but what if no underlying mental disorder can be demonstrated that requires treatment? It has been shown in the previous section that dissociation can occur in normal individuals as well. It has also been demonstrated that dissociation is a broad term involving many different causes and states. For this reason it is important to realize that dissociation is a psychiatric term, not a juridical one. The relevant legal concept is 'automatism'.

Automatism can be defined as a lack of control or involuntariness, but not as a lack of consciousness (3). Diminished consciousness can occur simultaneously but does not need to be present. The main difference between automatism and dissociation is that although automatism involves a state of dissociation, not all states of dissociation involve automatism. For example, if an individual simply cannot remember having committed a crime due to dissociative amnesia, this does not automatically mean he was unaccountable for it at the time. Automatism in the case of a non-premeditated intimate partner homicide mostly involves a dissociative fugue (3).

When the automatism defence is pleaded, two distinctions have to be made. First it has to be determined if there is an underlying mental disorder responsible for the dissociative state during a crime. If this is the case, it is categorized as 'mental disorder automatism' which attracts the special verdict 'not responsible on account of mental disorder' (3). If this is not the case, it has to be determined which subgroup of dissociation the crime concerns. This is relevant, because in case of intoxication, for example, the offender can still be held indirectly responsible for his crime. However, if no such external cause can be demonstrated, conviction will not take place (2).

Figure 1 - THE PROCESS OF EMOTION GENERATION IN EVERYDAY LIFE (20)



In summary it can be said that when a healthy individual without an underlying mental disorder and who is not under the influence of any substance becomes dissociated as a result of a 'psychological blow' -- and as a result suffers from a dissociative fugue during which a crime is committed -- the individual cannot be held accountable and will not be convicted.

Emotions

Dissociation can occur when a person is faced with an overwhelming and stressful experience (4). In the case of an emotion-induced dissociation it could be said that a person is literally being 'overwhelmed by his own emotions'. However, before the relationship between dissociation and mechanisms involving emotions can be discussed, it first has to be made clear what it is exactly that we call 'emotions'.

From an evolutionary point of view emotions are adaptive and therefore having them provides a survival benefit (18). They bring us into a state of readiness when we encounter a situation that threatens our own existence. Emotions also strengthen interpersonal relationships, social bonds and communication in general (19). Since humans are social beings, the ability to express and interpret emotions correctly enhances one's position in a group and thereby the chance to reproduce.

From a more philosophical point of view, emotions are part of what defines us as human beings. They enable us to experience a situation, to form an identity and to have affective relationships. Without emotions the world would be a grey place.

When analyzed, emotions are thought to consist of three components (19). First there is the subjective component because you can only experience an emotion. It cannot deliberately be chosen which emotion arises in a given situation. The situation is being evaluated and emotionally labelled without mental interference. Next there is the physical component consisting of the phenomenon known as arousal. This causes physiological changes associated with autonomic activity such as altered heart rate, sweating, trembling and increased skin conductance due to the release of catecholamines and glucocorticoids (16,19). Finally there is the cognitive component associating the emotional state with its source. This process is called attribution.

Emotions originate as a response to a certain stimulus, being either a thought or an environmental input. How the three components mentioned above integrate into an emotional reaction is not exactly known. An influential theory is the "two-factor" theory proposed by Schachter and Singer (1962). This theory states that physiological arousal and mental awareness of an emotion occur simultaneously and that the combination of the two is required to experience an emotion (Figure 1).

Findings that support this theory are the observation that arousal caused by a certain stimulus can be transferred to a new stimulus and thereby intensifies the latter (20). This is called excitation transfer. The main criticism of the theory is that the aspect of physiological arousal has been overstated, since there is no evidence that arousal is a necessary condition for an emotional state (20).

Decision-making

Besides providing a survival benefit and forming an essential part of what defines us as human beings, emotions also serve a valuable purpose in the process of decision making. At first this seems strange. It is tempting to assume that emotional and rational are two opposite extremes when it comes to making a decision. When facing a difficult situation one stays focused, thinks and makes effective decisions or one gets carried away by emotions, which generally results in more ineffective behaviour. Therefore it could be argued that it would be better to leave emotions out of the process of decision-making, since it is likely that they only disrupt it.

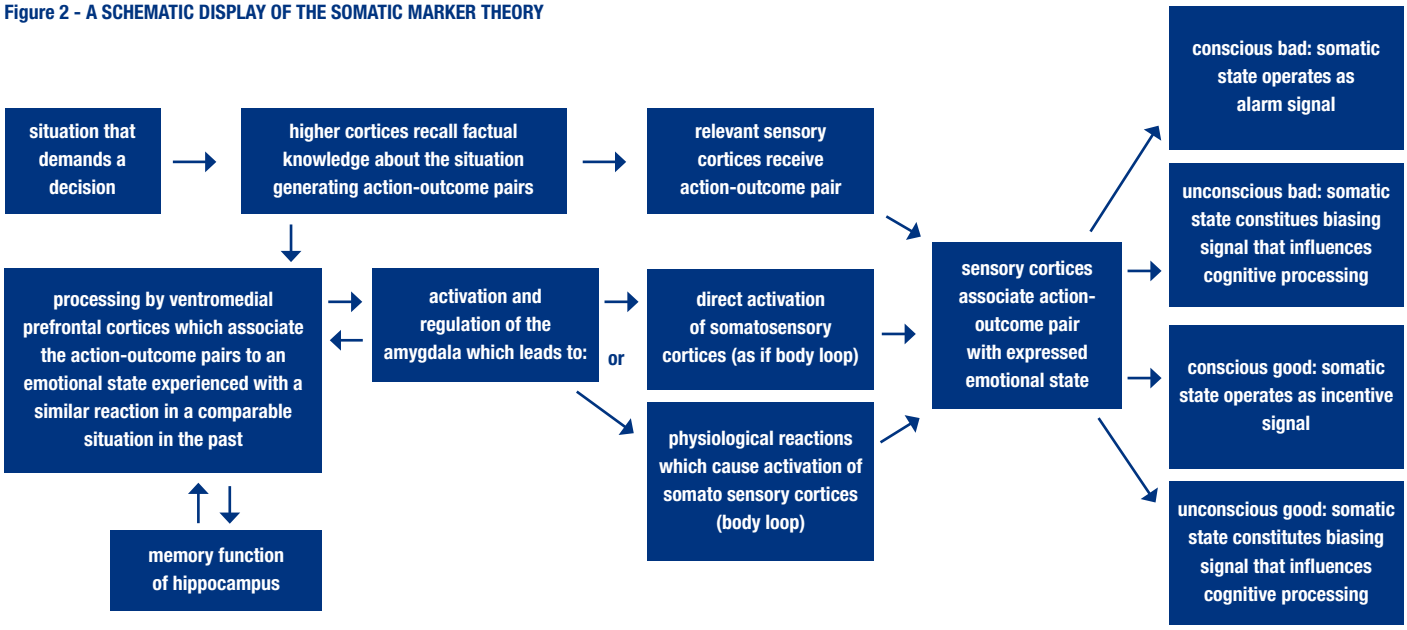
Strangely enough people that do leave the emotional component out of the decision-making process due to lesions in relevant brain structures like the ventromedial prefrontal cortices or amygdala do not function better than normal individuals, but worse (21). They suffer from a pathological condition known as alexithymia, which refers to the impairment of the ability to identify and communicate one's emotional state (22). Decision making in these patients is slower, more prone to error and fails to take into account previous experiences (23,24). They can also demonstrate impulsive behaviour and show little awareness of the moral implications of their actions (21).

Based on these findings it seems that in the decision-making process emotions, reasoning and logic are integrated into a system in which emotions form an essential foundation. The most influential theory explaining this system is somatic marker theory, proposed by Damasio in 1994 (Fig. 2). This theory is based on a number of lesion studies, clinical observations and skin conductance tests.

The somatic marker theory states that every time we face a situation in which a decision has to be made, several processes occur in the brain. First of all the factual knowledge about the situation is recalled and analyzed by higher order cortices in the brain. Possible actions and their consequences on both the immediate and longer term are being generated and projected onto the relevant cortices. Once an option-outcome pair is generated, these higher cortices also almost immediately activate the ventromedial prefrontal cortices, which include the orbitofrontal cortex (25). The ventromedial prefrontal cortices couple the received action-outcome pair with an emotional state experienced with a similar reaction in a comparable situation in the past. This process is aided by the memory function of the hippocampus (23).

Once the proper emotional state is connected to the action-outcome pair, the ventromedial prefrontal cortices activate and regulate the amygdala, which is capable of autonomic innervation. This is necessary in order to physically experience emotions (24). Supporting this concept is the observation that the ventromedial cortices have extensive bidirectional connections with both the hippocampus and the amygdala(21,23).

Figure 2 - A SCHEMATIC DISPLAY OF THE SOMATIC MARKER THEORY



The amygdala can express a particular emotional state in two different ways: through a 'body loop' or an 'as if body loop'. If a 'body loop' is followed the emotional state causes physiological reactions in the body, which in turn lead to activation of somatosensory cortices. If an 'as if body loop' is followed, somatosensory cortices are activated immediately by the amygdala. This pathway is quicker since it bypasses the body loop. However, as a result the emotional state experienced is much fainter compared to a body loop (25).
The somatic states experienced with the loops are called "somatic markers". They can manifest themselves consciously or unconsciously depending on the emotional load of the situation.

An 'as if body loop' is quicker and more efficient than a 'body loop' but creates less emotional awareness. This makes it more suitable for daily life decisions. On the other hand, extraordinary decisions are always accompanied by major emotional awareness as a result of the physiological changes caused by a 'body loop'.

The function of the 'loops' is to emotionally label the factual action-outcome options presented by the higher cortices. The somatic markers are being projected at the relevant somatosensory cortices simultaneously with the factual information. Based on the experienced emotional state, the projected factual knowledge is qualified as either good or bad. This system is advantageous, because stimuli containing an emotional load capture attention better than stimuli that do not (26). Therefore, the action-outcome pairs that are presented simultaneously with the somatic markers are being highlighted.

This means that an option-outcome pair can quickly be discarded or accepted, based on the emotion that is presented simultaneously. This makes the decision making process far more efficient and also enables people to make use of their own life experience.

If the somatic markers are absent, as in patients suffering from alexithymia, no action-outcome pair is being highlighted as either good or bad, and all options have to be cognitively analyzed. This explains the behaviour described in these patients earlier in this review.

It is interesting to examine the similarities between the Schachter theory on emotions and the somatic marker theory. When comparing the Figures 1 and 2, the somatic marker theory could almost be seen as a detailed version of the Schachter theory. With the described 'loop' mechanisms, it also deals with the main criticism of the Schachter theory. It might be suggested that the neurophysiological components and their interaction as described in the somatic marker theory not only have a function in decision making, but that they could also be responsible for inducing the right emotional state in a given situation.

Anger management

Now that a concept is present on how emotions are integrated in the decision-making process, the following point should be examined. Since we all experience emotions, why do some people get overwhelmed by them and others do not? In order to answer this question the process of emotion regulation has to be examined.

Different emotions influence our behaviour in different ways and sometimes also involve different brain structures (27). For this reason, emotions cannot be addressed in general here, but a single emotion must be specified. The most relevant emotion regarding emotion-induced aggression is anger, since this is the emotion most related to and responsible for reactive aggression (28). Another advantage is that anger is a basic emotion instead of a complex emotional state like jealousy, thereby making it easier to study.

There is an important distinction that has to be made when looking at anger. There is state-anger, which simply can be explained as what we experience at the moment we are angry, and there is trait-anger. Trait-anger involves stable individual differences in the frequency, duration, and intensity of state-anger (28). As could be expected, high-trait-anger individuals are more prone to state anger than low-trait-anger individuals (21). High levels of trait- anger are also associated with increased likelihood of alcohol and substance abuse, mental health problems, domestic violence and many other forms of aggression (29). All these factors are also risk factors for non-premeditated intimate partner homicide (30). Could it also be that high levels of trait-anger predispose an individual to dissociate? It has been demonstrated that extreme levels of anger are a central feature of many psychological disorders, including dissociative disorders (21,29). For this reason, it is interesting to examine the underlying mechanisms responsible for high levels of trait-anger.

The ventromedial prefrontal cortices are widely agreed to be the neural centre of executive function and effortful control of emotions, including anger (21). This view is supported by neuroimaging studies, lesion studies and the finding that high trait-anger individuals show diminished activity in these brain regions (31). Neuroimaging studies have shown activation of the orbitofrontal cortex during state-anger in healthy individuals (27). Also, specific damage to both the orbitofrontal cortex and the amygdala has been associated with impulsive and aggressive behaviour (21,23). Looking at this process on a smaller scale it is found that the relevant neurotransmitter used in the process of anger control by the ventromedial prefrontal cortices is serotonin (32). Therefore it is not surprising that reduced levels of serotonin are also associated with higher levels of trait-anger and reactive aggression (32).

Focussing on an even lower level, it has become clear that at the genetic level several genes are found that are proven to play a role in the regulation of serotonin levels. These genes are the TPH-gene (serotonin production), the 5-HTTLPK-gene (serotonin reuptake) and the infamous MAO-A-gene (serotonin degradation). Deficits in the expression of any one of these three genes is again associated with higher levels of trait-anger (32,33).

From a social-cognitive point of view, the most interesting finding is an attentional bias called rumination in high trait-anger individuals. Rumination can be defined as prolonged allocation of attention to negative information. High trait-anger individuals spend more attention over a longer period of time on a provocative stimulus, thereby prolonging the tendency to initiate state-anger (34).

Finally it is important to take into account the psychosocial development of an individual when looking at trait-anger. In line with the nature-nurture concept, the environment in which a person is raised also has a great influence on trait-anger and reactive aggression (28,29). Therefore research into exposure to aggression or emotional violence during life and especially during childhood is important when considering trait-anger (29).

It is interesting to see the amount of overlap between brain structures involving emotion regulation and decision making. Also, there is much resemblance between patients suffering from dissociative disorders and individuals who have high levels of trait-anger, especially during childhood development. However, from a psychological perspective it could be argued that high trait-anger individuals should dissociate less often than normal individuals. Since they experience anger more often and more intensely, they should be accustomed to it and should not get dissociated as a result of emotional stress. This could be compared with bungee jumping. The first time you jump, you might dissociate because it feels as if you are about to die, but when you go bungee jumping every weekend the tendency to dissociate that you had on your first jump is less likely to occur.

This would suggest a larger physiological involvement in the cause of dissociation. An explanation could be that high trait-anger individuals experience more emotional stress, because they are more often in an emotional state. This means that their brain is more often exposed to catecholamines and glucocorticoids that are released as a result of autonomic activation (16,19). High concentrations of these substances have a particularly adverse effect on the hippocampus, because it contains high numbers of glucocorticoids receptors (35). Large doses of catecholamines and glucocorticoid also have an adverse effect on the human brain in general and its functional integrity (36,35). These adverse effects might favour the occurrence of dissociation.

Discussion

Now that an understanding of dissociation, emotions and decision-making is present, a theory can be proposed that might answer the question why people can act impulsively, violently and even murderously in a state of emotion-induced dissociation.

The information present in current literature could suggest the following: When an individual reaches an emotional state, autonomic activation occurs. This is caused by the interaction of brain structures that are also relevant in the decision making process as described in Figure 2.

As a result, glucocorticoids and catecholamines are released, causing physiological reactions that lead to physical experience of the emotional state. Problems arise when individuals are unable to adequately regulate their emotional state. This leads to a more intense emotional state and subsequently to higher circulating levels of catecholamines and glucocorticoids. The high concentrations of these substances can cause a temporary dysfunction in the hippocampus (35). As a result, a dysfunction occurs in the ventromedial prefrontal cortices, because suddenly no memory of emotional states is present. Since no emotional input is received from the hippocampus, the ventromedial prefrontal cortices stop activating the amygdala and down regulate its activity.

The dysfunction of the interaction between these brain structures causes 'emotional numbing' since the amygdala no longer causes autonomic innervation and no new emotional input is received by the ventromedial prefrontal cortices. This could be seen as a beneficial feedback mechanism, because as a result the circulating levels of catecholamines and glucocorticoids will be reduced. This protects the brain from a toxic degree of exposure to these substances.

The abrupt emotional numbing might lead to experiences of depersonalization, since this phenomenon is also described the other way around (15,16). Together with the impaired memory function of the affected hippocampus (36), this could be responsible for the experience of a dissociative fugue.

Then why do people act impulsively and violently in this state? This might be contributed to the emotional numbing. When the ability to experience emotions is compromised, it is logical to assume that the second function of experiencing emotions, namely their role in decision making, is compromised as well. As a result, decision making can become impulsive, without any regard for the consequences. This is supported by the observation of patients who suffer from lesions in amygdala and ventromedial prefrontal cortices. They demonstrate exactly this type of impulsive behaviour and also show little awareness of the moral implications of their actions (21).

Conclusion

Imagine yourself again coming home unexpectedly, as in the introduction. The impulse to kill your wife when catching her in bed with your best friend might also arise in a normal and non-dissociative state, but is not executed because of the emotional awareness and moral understanding that this option-outcome pair is wrong and not beneficial at all in the long term. However, in a state of dissociation this 'emotional brake' that normally prevents a person from initiating unwanted behaviour is disabled.

It is difficult to murder another human being if you are not in a state of dissociation and it could be considered unlikely that an adaptive trait like emotions would favour impulsive murderous behaviour.

In conclusion it could therefore be argued that rather than murdering your wife in an 'emotional frenzy' as one would expect, the experienced emotional arousal causes a neural dysfunction that leads to the very opposite: a cold-blooded murder without any emotions to restrain the murderer.

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Instructions for EJM authors

General

The instructions that follow have several purposes. First, we want to make life easy for you, the authors, and for the editors and peer reviewers, the layout (prepress) people, and the journal readers.

The Guidelines for the Storyline will help you to organize your article in a logical, credible and readable way. This will help you—it tells you what goes where—and, thus, save you time. It will help the editors and peer reviewers—they will easily see the credibility and relevance of your work— and, thus, save them from writing rejection letters. And, it will help readers to quickly and easily read and understand your work and see its value.

The section entitled Formatting Instructions will help you as well; the basic idea is to keep the formatting as simple as possible, so you can focus on content and not get involved with layout. The language editor and the prepress people will also be able to more efficiently do their jobs. Please follow these instructions. Please be aware that we will have to return papers that do not conform to these instructions to the authors.

What you can enter

Research articles - Research articles describe one study or analysis, usually from a fourth-year elective research project or one of the masters programs. Number of words: max. 3500 + 4 figures or tables.

Extended abstracts - Extended abstracts consist of a condensed presentation of complete final or temporary results of a study. Number of words: 350 words + 1 figure or table.

Research papers - Here researchers or teachers describe ongoing research projects at the Erasmus Medical centre for which they want to invite students to participate. Number of words: 350.

Reviews - Second year students can submit their review written in the second year elective course. Number of words: 1500 + 3 figures or tables

Opinion papers - These are papers that reflect the opinion of the author on a scientific topic. The author should be clear where evidence ends and personal opinion starts. A paper typically has a length of about 1000 words.

Comments - In this section editors, or faculty staff, as well students are invited to write a short critical comment on a paper, putting it into perspective for a broader medical public readership. Number of words: 350.

Letters to the editor - The editorial board encourages students to write a letter to the editor to comment on published papers, or on the journal in general. These will be published on the website of the journal. Letters should not exceed 200 words and may be abbreviated by the editor.

Formatting instructions

Entry format - Papers should be submitted by email, to ejm@erasmusmc.nl. Word 2003 files are preferred for the initial submission. The file should include all figures and tables.

Title page - The title page should clearly identify the authors, the institute where the research project was carried out, as well as the staff member who supervised the project. The corresponding author name (first name and family name), email address, student id, should be clearly indicated. In case of multiple authors, state functions and departments only in superscript in alfabetical values. Example:

First name A.G. Family name[a] and First name W.F. Family name[a]
Supervisor: First name R. Lastname[b]

[a] Medical students, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Dept. of Internal Medicine, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: First name A.G. Family name, email: Firstname-Familyname@me.com.

Structure - Please use the following sections in all papers (except in comments and opinion papers): Abstract, Introduction, Methods, Results, Discussion, References, Tables, Figures.

References - Number references in order of appearance. References should have the following format:
Rothwell, P. M. Medical and surgical management of symptomatic carotid stenosis. Int.J.Stroke. 2006; 1: 140-149. (I.e. year;vol:ppp-ppp)
In case of more than 3 authors, name the first 3 and insert "et al.".
Limit the number of references to 30. References should appear in the text as follows "….treatment is of proven benefit.1"

Tables and figures - Tables and illustrations (both numbered in Arabic numerals) should be prepared on separate pages. Number tables and figures separately and consecutively. Tables require a heading and figures a legend, also prepared on a separate page and should be formatted with a text editor (example). Figures should be submitted electronically. B/w half-tone and color illustrations must have a final resolution of 300 dpi after scaling, line drawings one of 800-1,200 dpi (jpg and tiff is an acceptable format). Please note that all color-figures will be converted to gray tones. Please adapt graphs to suit this format, i.e. make use of dotted and dashed lines and hatched bars instead of colored items.. The final submission should contain figures as JPG or TIFF files.

Page layout

- Standard margins
- no headers or footers
- no columns
- left align (ragged right)
- font: 12pt Arial
- single line spacing
- main headings 14 pt bold; subheading 12 point italic
- indent every paragraph, except after headings, tables, bulleted lists or figures

Other formatting

- number all tables and figures sequentially
- place tables and figures at the end of article; insert captions at correct locations in body text
- no text boxes
- no footnotes or end notes
- do not submit figures with text as drawing objects (they cannot be edited)
- limit the use of italics and do not use italics for simple emphasis; do not italicize quotations; quotation marks are sufficient
- do not use italics for commonly understood Latin expressions such as "in vitro"
- use italics for other foreign words, such as expressions in Dutch
- no "sub-paragraphs"
- no hyphenation (afbreking)

Language

US English spelling and punctuation

Guidelines for the storyline of a scientific article

Authors of scientific articles often write in a style that was very successful at school but, unfortunately, does not work well in the "real" world. The reason? Readers of scientific journals -"real world readers" - when reading your papers have much different goals than your instructors had. Real world readers do not care how smart you are, or if you have done the homework, and they are not going to "grade" your papers. They do not count words to see if you met the minimum length of the paper. They read journal articles because they are looking for information and ideas that they can use in their own work. In other words, they are looking for something of value. In fact, when you read journal articles you are also a real world reader. Do you care how smart the authors are, or if they did their homework? Probably not. Are you impressed with long complicated sentence constructions and abstract ideas that only seem to fill up space? Do you count words? Probably not.

You probably are impressed, however, when you read an easy to understand article that clearly presents credible and relevant science. You can write that way too. But you need to get "out of the box" of what I call "academic" writing style. These guidelines will help you to get outside the box. The guidelines are based on one very simple concept: give you readers something they can use. Note that this requires a different mode of thinking. At school you probably never thought of giving your instructors something they could use. You wrote to receive something - a good grade. These guidelines will take you step by step through the process of writing a readable scientific article that presents relevant and credible science.

The first step is to write the "storyline" of your article - a readable story that logically ties all of your main ideas together. It focuses on the logical thread that credibly presents the point and the value of your research. It is to be short, logically linked and easily understood by non-specialists. It contains few technical or theoretical details. This requires another shift in your mode of thinking. It requires you to forget, for the moment, the technical and theoretical details and problems that you focus on day-in and day-out. Rather, you need to think in terms of what the reader wants to know - the point and value of your research.

Your storyline will become a "skeleton" for your entire article. After getting the storyline clear, take the second step and add the "muscles" to turn this skeleton into a complete entity - a readable and credible scientific article. The "muscles" are, of course, the technical and theoretical details.

Below, is a short (fictitious) example to illustrate the storyline. In this example, 6 key elements of the standard scientific article form the basic structure of the storyline. You can use this example as a template for your storyline. Under each heading, delete the example text and paste in a similar text about your own work.

Fictitious Example

"Predicting Malaria Epidemics in Ethiopia"

Key element 1: the point of the research - why should the editors and readers care about the study?

'Malaria is still the number one killer of all infectious diseases. Most deaths could be prevented, however, if adequate medical facilities and medicines were available at the beginning of an epidemic. After an outbreak of malaria, getting adequate medical facilities and medicines to the local area can take many weeks. Obviously, this time is truly lost time and, for many victims, fatal. If, however, malaria epidemics could be predicted in local areas, medical facilities and medicine could be mobilized where they will be needed and, thereby, save many lives. Predicting where and when an epidemic can be expected is, however, currently not possible.'

Notice that the above statements clearly present a BIG health-related problem. And, an "if" sentence focuses on a strategy to help solve that problem. In only 3 sentences we know the point, and potential value, of the research. Now it is time to focus on what is known, and prepare the reader to understand the specific research question. For example,

'Malaria epidemics are known to be related to weather conditions. Previous research has shown that malaria epidemics seem to be related to specific meteorological factors (refs.). Smith and Jones (1995) have shown... Adams (1997) found that ... The correlations between these meteorological factors and subsequent malaria epidemics, however, have never been systematically investigated. If such correlations do indeed exist, meteorological factors might be used to predict local epidemics. In this study we take a first step in developing a predicting model.'

At this point, the reader should have a good idea of the focus of the research. Now it is time to "zero in" on the specific research questions.

Key element 2: the specific research questions - the basis of credible science

'The purpose of this study was to answer the following questions. (1) What retrospective meteorological factors, and what combinations of factors, correlate significantly with the occurrence of subsequent malaria epidemics in Ethiopia? (2) To what extent do they explain the variance of occurrence of subsequent epidemics?'

Notice that the research questions are stated in terms of the variables that were measures or observed, in this case, meteorological factors (the independent variables) and occurrence of epidemics (dependent/outcome variable). Furthermore, the questions state the relationships sought between the variables: correlations and explanation of variance of the dependent variable. Such specific research questions tie the story together—they focus on credible science.

Key element 3: a description of the methods you used to answer your research questions.

This section will later become your Methods section. For the storyline, avoid details and make it understandable to the non-expert. Note that it is in past tense - factual information about what you did in this study.

'In a retrospective study, we collected meteorological data for 10 local areas in Ethiopia. The data included rainfall, temperature, sunshine, AAA, BBB, CCC, and DDD and... We also collected data concerning malaria epidemics for the same areas. This data covered the years 1963 to 2006. We developed a statistical model to determine correlations, and find factors and combinations of factors explaining the variance of epidemics. Using an independent subset of the data collected, we determined the predictive power of the model.'

Notice that in this section the authors report 2 types of information: (1) how they collected data, and (2) how they determined relationship between the variables.

Instructions for EJM authors

Key element 4: the major findings
This will later become your Results section.
'We found that factors AAA, BBB, and CCC correlated significantly with subsequent epidemics in all 10 of the local areas studied. In 3 of the areas, the combination of CCC and DDD correlated significantly.'
Notice that in this section the authors report the relationships between the variables that they found. These are historical facts and, therefore, reported in past tense.

Key element 5: the answers to the research questions - your interpretation of the factual findings.
This will become the beginning of your Discussion section.
Notice that the answer to the research question uses exactly the same words used to state the question. And, notice that it is not a summary of results, but the authors' interpretation of the results about how the world IS and, therefore, stated in present tense. Of course, in a pilot study such as this, the authors cannot yet present definitive answers, and they indicate that with the words "suggest" and "may."
'The results of our study suggest that factors AAA, BBB, and CCC correlate significantly with subsequent malaria epidemics in Ethiopia. Furthermore, the combination of factors CCC and DDD may account for about XX% of variance in some areas. If we can generalize our findings to other areas, our model will have a predictive power of...'

Key element 6: the consequences of the answers—the value of your work.
This will become the Conclusion section and it relates directly back to the first key element, the original big health-related problem. A Conclusion is NOT a summary of results, but it describes how the study helps to solve the problem—it ties the end back to the beginning. And, it suggests a next step toward solving the problem - it gives direction to research.

'We conclude that local meteorological data can be used to predict malaria epidemics. Our statistical model, developed in this pilot study, has a predictive power of about 30%. Although this is certainly a first step toward predicting malaria epidemics, we would like to considerably increase the predictive power. We think that inclusion of groundwater level might increase the model's predictive power. This factor is, however, not available in the databases we used and will have to be determined by other means. Furthermore, our model still needs to be validated in other areas.'

The example as running text - a stand-alone story that focuses on the point and value of the research.

'Predicting Malaria Epidemics in Ethiopia'

Introduction
Malaria is still the number one killer of all infectious diseases. Most deaths could be prevented, however, if adequate medical facilities and medicines were available at the beginning of an epidemic. After an outbreak of malaria, getting adequate medical facilities and medicines to the local area can take many weeks. Obviously, this time is truly lost time and, for many victims, fatal. If, however, malaria epidemics could be predicted in local areas, medical facilities and medicine could be mobilized where they will be needed and, thereby, save many lives. Predicting where and when an epidemic can be expected is, however, currently not possible. Malaria epidemics are known to be related to weather conditions. Previous research has shown that malaria epidemics seem to be related to specific meteorological factors. The correlations between these meteorological factors and subsequent malaria epidemics, however, have never been systematically investigated.
The purpose of this study was to answer the following questions. What retrospective meteorological factors, and what combinations of factors, correlate significantly with the occurrence of subsequent malaria epidemics in Ethiopia? To what extent do they explain the variance of occurrence of subsequent epidemics?

Methods
In a retrospective study, we collected meteorological data for 10 local areas in Ethiopia. The data included rainfall, temperature, sunshine, AAA, BBB, CCC, and DDD and... We also collected data concerning malaria epidemics for the same areas. This data covered the years 1963 to 2006. We developed a statistical model to determine correlations, and find factors and combinations of factors explaining the variance of epidemics. Using an independent subset of the data collected, we determined the predictive power of the model.

Results
We found that factors AAA, BBB, and CCC correlated significantly with subsequent epidemics in all 10 of the local areas studied. In 3 of the areas, the combination of CCC and DDD explained XX% of the variance in occurrence of subsequent epidemics.

Discussion
The results of our study suggest that factors AAA, BBB, and CCC correlate significantly with subsequent malaria epidemics in Ethiopia. Furthermore, the combination of factors CCC and DDD may account for about XX% of variance in some areas. If we can generalize our findings to other areas, our model will have a predictive power of about 30%.

Conclusion
We conclude that local meteorological data can be used to predict malaria epidemics. Our statistical model, developed in this pilot study, has a predictive power of about 30%. Although this is certainly a first step toward predicting malaria epidemics, we would like to considerably increase the predictive power. We think that inclusion of groundwater level might increase the model's predictive power. This factor is, however, not available in the databases we used and will have to be determined by other means. Furthermore, our model still needs to be validated in other areas.

As a running text, it is now a short (452 words) and understandable story that forms the skeleton for the journal article. All we need to do now is to fill in the scientific and technical details - without destroying the storyline. To ensure that your article clearly presents the point of your research, write a similar short storyline for your study. Then fill in the details (theory, references, methods, data, tables, figures etc.) needed to support that storyline.

Do not underestimate the difficulty of getting out of that box of technical details. The author of the above example was also in a box, a box full of complex statistical methods and computer algorithms to design his model. And, as a consequence, he had lost all sight of the health-related problem he was helping to solve - malaria. I hope this example will help you to get started.

- Ed Hull -

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