The autonomic nervous system plays a major role in maintaining homeostasis. Short-term blood pressure and heart rate control are mainly modulated by the autonomic nervous system. Therefore, autonomic function can be assessed by cardiovascular autonomic function tests. As mentioned in the **introduction**, a battery of tests is usually performed to determine autonomic function. Some of the tests, or parameters derived from a test or measurement, provide insight in sympathetic function, others in parasympathetic function and some in sympathetic as well parasympathetic function. The background and methodology of all tests used in this thesis are mentioned in the **introduction**.

The cold face test

In humans, it has been established that diving results in bradycardia and peripheral vasoconstriction. Previous studies have shown that the diving reflex can be elicited by the application of a cold stimulus to the face. Our interest in this test was based on the ability to assess simultaneously, but separately, parasympathetic and sympathetic function. This is in contrast to many other tests in which the parasympathetic and sympathetic reaction can not be distinguished or assessed simultaneously. In **chapter 2** we propose a methodology to assess the cold face test, taking the ability to breathe and ocular pressure, which can induce the oculocardiac reflex, into consideration. With an intact parasympathetic nervous system, variation in intrathoracic pressure and central neural modulation, as induced by breathing, result in heart rate changes. We found that the heart rate decrease was more outspoken during the application of a cold stimulus, a plastic bag filled with melting ice-water at 0ºC, when a subject was unable to breathe. The importance of the oculocardiac reflex was investigated by varying the mass of the plastic bag, which provided the cold stimulus, and by the wear of diving goggles. As we found no differences in heart rate or blood pressure changes between the various masses or by wearing diving goggles, it seems that the ocular pressure during the cold face test is too small to elicit the oculocardiac reflex. We propose to perform the cold face test by covering the whole face with a plastic bag filled with melting ice-water, whereby the subject is not able to breathe. The mass of the plastic bag does not seem to be important, but it should be large enough to cover the whole face of the subject. As repeatability was improved by habituation to the cold stimulus, our results make it advisable to practise the test before actually assessing the diving reflex. The heart rate decrease may be more pronounced during longer application of the cold stimulus. As subjects seem to be able to sustain the stress for 40 seconds when encouraged, we suggest this as test duration. Future study is needed to reveal whether the decrease in heart rate during the test is related to the age of the subject, the duration of the test, or the combination. Age-related normal values have to be established.

We wondered whether the cold face test, as described in **chapter 2**, is a useful tool in detecting changes in autonomic function. Digoxin or enalapril in healthy subjects modulated autonomic function. Digoxin is known to result in a slowing of the heart rate, which, apart from direct effects on the AV-node, is partly ascribed to augmentation of vagal activity. Enalapril and other ACE-inhibitors are known to reduce sympathetic tone. This contributes to the fall in blood pressure without a change in heart rate. Little is known about a possible facilitation of vagal tone by ACE-inhibitors. **Chapter 3** describes a placebo-controlled cross-over study, where the cold face test was assessed after treatment with placebo, digoxin or enalapril in 11 subjects. To measure peripheral blood flow, venous occlusion plethysmography was performed during the cold face tests. We found that baseline heart rate was lower when subjects had taken digoxin compared to placebo. However, no differences in heart rate decrease between the different treatments were found. This suggests that the cold face test stimulates vagal activity maximally, so that the heart rate decrease can not be more pronounced. As pointed out in **chapter 2**, the decrease in heart rate might be influenced by the duration of the test. In the study described in **chapter 3**, subjects were able to sustain the test till maximum endurance. When subjects were treated with enalapril the duration of the cold face test was significantly shorter compared to placebo. This might reveal a facilitatory activation of vagal tone by enalapril. During treatment with enalapril the blood pressure rose less in comparison to placebo. Surprisingly, a lower vascular resistance in our study could not explain this. This may be due to the technique used: blood flow was measured at the calves, whereas forearm plethysmography is mostly used.

From our studies we conclude that the cold face test is such a strong stimulus that even when it is sustained shortly a strong parasympathetic response is provoked. The test seems not suitable to detect (small) changes in autonomic function.

In conclusion, in terms of an efficacy model, **chapter 2** defines some prerequisites for the first step in such a model: the assessment of technologic capability. **Chapter 3** illustrates that the cold face test, despite the pathophysiologic insight it may provide, is too limited in its possible use. The effect of an intervention or the modulation of the autonomic nervous system can not be determined by it. Therefore, it does not pass beyond the second step in the efficacy model.

Amyloidosis

Patients with systemic amyloidosis usually develop a severe autonomic dysfunction during the course of the disease. Therefore, it is an excellent population to study autonomic function tests. However, patients with rheumatoid arthritis, which is usually the cause of AA amyloidosis nowadays,

can experience difficulties in performing cardiovascular autonomic function tests such as the isometric handgrip test (by lack of strength). Due to the severe autonomic dysfunction, some patients with systemic amyloidosis have such orthostatic hypotension that they can not stand. In these patients it may be difficult to define autonomic function by the tests as proposed by Ewing and Clarke (Br Med J 1982; 285: 916-8). In **chapter 4** we describe how autonomic function was defined in 19 patients with AA amyloidosis, 21 patients with AL amyloidosis and 6 patients with ATTR amyloidosis. Only 28 patients were able to perform all tests as proposed by Ewing and Clarke (deep breathing, Valsalva manoeuvre, isometric handgrip and standing up). Especially patients with AA amyloidosis were unable to perform all 4 tests: only 5 out of 19 patients.
Of the remaining patients, 6 had such abnormal test results on the few tests they could perform, that it was obvious they had definite autonomic failure. Besides the Ewing tests, the mental arithmetic stress test, the cold pressor test and head-up tilting were performed. The mental arithmetic stress test and the cold pressor test induce a sympathetic reaction which is similar to the isometric handgrip test. Eight patients did not tolerate the cold pressor test, while all patients could perform the mental arithmetic stress test. By taking the mental arithmetic stress test and head-up tilting into consideration, autonomic function could be defined in 11 of the remaining 12 patients.
Of the 19 patients with AA amyloidosis, 8 patients were found to have severe autonomic neuropathy, which is more than expected from the data in the literature. Whether the autonomic dysfunction is due to the amyloidosis or to the rheumatoid arthritis remains an unresolved issue.
Cardiac involvement of the amyloidosis is frequently encountered. This may result in an impaired effector function of the heart. Cardiac involvement was studied by measuring the mean left ventricular wall thickness by echocardiography. In our study, patients with a mean left ventricular wall thickness >12 mm, which implies cardiac involvement of the amyloidosis, did not have more frequently autonomic failure than the patients with no signs of cardiac involvement. This suggests that cardiovascular reflex tests may be performed in patients with cardiac amyloidosis. However, the results should be interpreted with caution as heart failure itself leads to changes in autonomic function.
Thus, **chapter 4** is an illustration of the use of the concept of clinical effectiveness. Even the 'standard' of cardiovascular autonomic function tests is not feasible in less than ideal conditions. Heart rate variability is an alternative method to assess autonomic function. Because it is less dependent on the possibility and willingness of a patient to co-operate, it may perform better in an assessment of its clinical effectiveness. In patients with a myocardial infarction, with chronic heart failure and in the general population, the standard deviation of all normal to normal RR-intervals (SDNN) can predict mortality. In **chapter**

5 we describe that 1-year mortality can be predicted by the SDNN measured during 24 hours. Patients with AL amyloidosis and a SDNN ≤50 ms were found to have a 22-fold (95% CI 5.4-90.4) increased risk to die within 1 year compared to patients with a SDNN >50ms. Previously, the mean left ventricular wall thickness was found to predict mortality in patients with AL amyloidosis. We found that both measurements are complementary prognostic indicators. The study by Dekker and colleagues (Circulation 2000; 102: 1239-44) showed that a 2-minute rhythm strip can predict mortality in healthy persons. If this would be equally predictive as the 24-hour standard deviation of all normal to normal RR-intervals is in our study, it would signify an important progress in the clinical effectiveness. Such a measurement would warrant further assessment of clinical efficacy.

The baroreflex sensitivity is a parameter of autonomic function which takes not only heart rate variation, but blood pressure variation into consideration also. This may be a better parameter to define autonomic function than heart rate variability, as has been suggested for patients with a myocardial infarction and patients with diabetes mellitus. Therefore, it might be a better prognostic indicator. In **chapter 6** the prognostic value of the standard deviation of all normal to normal RR-intervals and the baroreflex sensitivity are compared. The baroreflex was not found to be a predictor of 1-year mortality in this study. Whether this is due to the small group of patients, the technique used or that the baroreflex sensitivity is not only a parameter of neural activity remains to be elucidated. Although the study population was smaller than in the previous study, the SDNN remained a predictor of mortality in patients with AL amyloidosis.

Heart transplantation

After transplantation, the donor heart is denervated as all cardiac nerves are severed during the orthotopic heart transplantation. In previous studies, reinnervation of the parasympathetic as well the sympathetic nervous system has been documented. How long after heart transplantation and in which sequence parasympathetic and sympathetic reinnervation takes place has not been revealed. **Chapter 7** describes 16 patients, 1-97 months after heart transplantation, in whom autonomic function was assessed. The results of their cardiovascular autonomic function tests were compared with the results of 16 age and sex matched controls. Our results suggest that parasympathetic reinnervation precedes or accompanies sympathetic reinnervation. More transplant recipients had normal outcomes at parasympathetic tests than at sympathetic stress tests, and none had normal sympathetic test results without normal parasympathetic test outcome. As found in other studies, a younger age of the donor was associated with reinnervation.

Recently, Bengel and colleagues demonstrated that sympathetic reinnervation results not only in an improved heart rate response, but also in an improved contractility of the heart during exercise (NEJM 2001; 345: 731-8). Patients with sympathetic reinnervation after cardiac transplantation had a significantly better exercise performance than the patients without signs of reinnervation. Until now, it remains unresolved after which time interval reinnervation occurs and when it is maximal. To answer these issues, autonomic function should be assessed in patients undergoing cardiac transplantation prospectively and longitudinally.

Conclusions

By measuring heart rate and blood pressure during cardiovascular tests autonomic function can be determined. Skill is still needed to select the tests to answer what you are looking for in a specific patient. Although normal values have been proposed, each laboratory, which starts to perform cardiovascular autonomic function tests, should allow a period to practise the tests and to assess their reproducibility.

In this thesis, a methodology for the assessment of the cold face test is proposed. The application of an ice-cold stimulus to the face was found to evoke a strong parasympathetic reaction. As a result, the cold face test seems not suitable to detect subtle changes in autonomic function. The test can best be performed after habituation to the stimulus, while the subject is not able to breathe.

Although patients with systemic amyloidosis can not perform all cardiovascular tests as recommended by Ewing and Clarke, autonomic function can be defined in the majority of these patients when assessing the mental arithmetic stress test and head-up tilting also. The standard deviation of all normal to normal RR-intervals, and not the baroreflex sensitivity, was found to predict 1-year mortality in patients with (AL) amyloidosis.

Autonomic reinnervation occurs in patients after cardiac transplantation. It seems to be related to the age of the donor heart and the time after transplantation. We found that sympathetic reinnervation was always accompanied by parasympathetic reinnervation.

In conclusion, cardiovascular autonomic function tests still have a long way to go along the path of clinical efficacy assessment.