1 Introduction

Within the general population and especially western population (Europe and American), infections and parasitic diseases and diseases of the cardiovascular system are the leading cause of death, followed by malignant tumors (WHO report 2000, Figure 1.1).

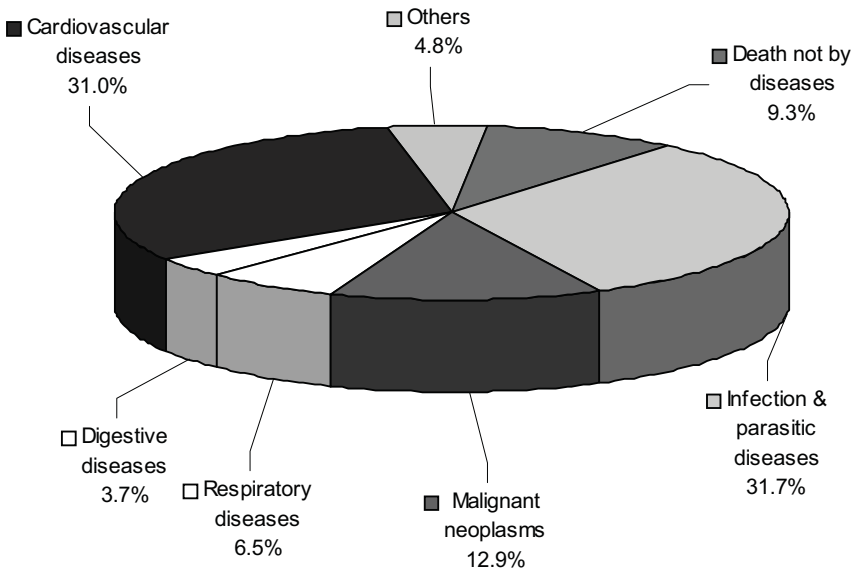

Figure 1.1 Cause of death in the general population in year 1999 (WHO report 2000)

Epidemiological research during the last twenty years emphasizes some risk factors for cardiovascular diseases: hyperlipidemia, hypercholesterolemia, smoking, high blood pressure, obesity and diabetes. Smoking cessation, reduction in LDL-cholesterol serum concentrations and normalization of blood pressure have been shown to be effective strategies in the prevention of cardiovascular disease (CVD). However, these major classic cardiovascular risk factors and such non-modifiable risk factors as age, sex and family history cannot fully explain why some persons develop myocardial infarction and/or stroke while others do not. Other factors may also increase the likelihood of developing cardiovascular diseases and may contribute to atherogenesis. Pathologic and epidemiological studies suggest that only about one half to two thirds of the variation in the anatomic extent of atherosclerosis and risk for atherosclerotic vascular disease can be explained by classic risk factors.

Therefore, many emerging risk factors have been found and among these, elevated plasma tHcy concentration (hyperhomocysteinemia) has been designated as a new risk factor for cardiovascular disease.

Homocysteine is a naturally sulfur containing amino acid which is generated as an intermediate product in the methionine metabolism, an essential dietary amino acid. Hyperhomocysteinemia is generally defined as a state when fasting plasma total homocysteine concentrations exceed concentrations of 15 μmol/l. This has been shown to be an independent risk factor for cardiovascular disease. However, there are many factors which directly or indirectly influence the concentration of tHcy such as genetic defects, vitamin deficiency, life style, disease and medication.

Since homocysteine is associated with the development of atherosclerosis and vascular disease, there is growing interest in highly specific and sensitive methods for the detection, quantification and progress of increased concentrations of tHcy in many clinical cases.

Generally, it is the aim of this dissertation to develop a sensitive and accurate new gas chromatographic-mass spectrometric (GC-MS) method for the determination of homocysteine, which also allows to determine simultaneously other related amino acids like methionine, cysteine and cystathionine. To validate this method we compared the results with those of an established high-performance liquid chromatography (HPLC) method and IMx homocysteine assay, and we applied this new GC-MS method in two clinical investigations. We targeted our study at patients with end-stage renal disease (ESRD), which is known to carry high risks for cardiovascular disease. In detail, the specific objectives were :

- To develop a GC-MS method for determination of tHcy and related amino acids like methionine, cysteine and cystathionine.
- To compare this new GC-MS method for determination of tHcy with an established HPLC method and an IMx homocysteine assay.
- To apply this GC-MS method on hemodialysis patients in order to observe the metabolism of homocysteine on ESRD patients and its relationship with related amino acids.
- To compare effects of Leucovorin (N^5-formyltetrahydrofolate) versus folic acid in reducing plasma tHcy in ESRD patients. Additionally, we observed the concentrations of the other related amino acids under this therapy.

In chapter 2, a short overview on the theme of homocysteine is presented describing a theoretical background of homocysteine including history, metabolism, determinants and its relationship with cardiovascular and renal disease.

In chapter 3, the development of the new GC-MS method using isotope dilution method is described, including the analytical performance of the method.

In chapter 4, the comparison of the new GC-MS method with an established HPLC method and IMx homocysteine assay is described.

In chapter 5, the application of this method in two clinical investigations is presented. In the first study we observed the homocyteine metabolism in ESRD patients, and in another study we compared the effects of Leucovorin versus folic acid in reducing plasma tHcy and the other metabolites in ESRD patients.

Finally, we discuss our findings in chapter 6 by comparing these with previous results from other research groups.