A fascinating scientific journey with randomized clinical trials - with translational “gold” in corresponding biobanks for the future

Professor emeritus

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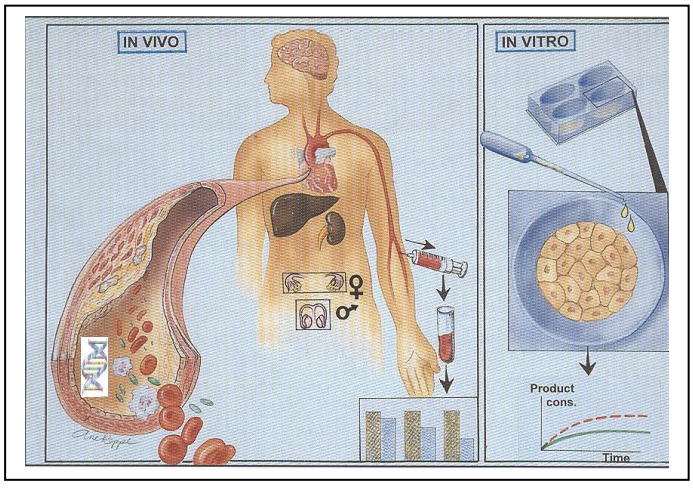

It all started in 1968 when I was asked by dr. Sverre Blix, a pioneer in **fibrinolytic research** with his "euglobulin clot lysis time" for quantification of fibrinolytic activity, to take on responsibility for the Norwegian part of an international study on the effect of urokinase in patients with an acute myocardial infarction. As a junior assistant doctor at the Department of Cardiology at Ullevål university hospital I was attracted by the possibility to perform clinical research with backing from the already well-known Haematological Research Laboratory under supervision from professor Hans Christian Godal.

With a scholarship from Nasjonalforeningen for Folkehelsen, Det Norske Råd for Hjerte- og Karsykdommer altogether 81/341 patients were included by us, 40/172 patients treated with urokinase. The main study was published in Lancet in 1975 with a completely neutral clinical result, probably because of too long time from symptom start to treatment (18 hours) *("Controlled trial of urokinase in myocardial infarction. A European Collaborative Study." The Lancet October 4, 1975, p.624).* However, preparation of fresh samples from the 40 urokinase treated patients introduced the opportunity for *"Studies on Fibrinogen/Fibrin Degradation Products. With special reference to characterization, anticoagulant activity and influence on laboratory assays",* which was the title of my Thesis being created as a Dr.Med. at University of Oslo in 1974.

RCT on venous thromboembolism

In Hematological Research Laboratory we were more concerned with venous thromboembolism, and being clinically fascinated by RCTs focus was now shifted to deep venous thrombosis (DVT) and pulmonary embolism (PE). In 1978 we published *"A Prospective Study of Streptokinase and Heparin in the Treatment of Deep Vein Thrombosis" (Acta Med Scand 1978; 203: 457)*. Altogether 42 patients with extensive thrombosis beyond the calf veins were included. Significant thrombolysis occurred in 71.4% of 21 patients treated with streptokinase and in 23.8% of the 21 heparin-treated patients, a highly significant difference ($p=0.002$). It was concluded that patients with acute DVT with proximal extension of the thrombus beyond the calf veins should be offered a therapeutic trial with streptokinase.

In 1982 we published the follow-up of these DVT patients after 6½ years. Seven patients had died, thus 35 patients were subjected to the follow-up study consisting of phlepography and clinical examination. Seven patients had phlebographically normal veins, and all belonged to the streptokinase group. The difference between the treatment groups was statistically highly significant ($p<0.01$). At clinical examination, 13 of the 17 patients in the streptokinase group had normal legs and 4 exhibited moderate postthrombotic changes. In contrast, 3 of the heparin-treated patients showed serious postthrombotic changes with open leg ulcers, and only 6 of 18 patients in this group had normal legs. It was concluded that streptokinase therapy is the best treatment at present in patients with acute DVT. This has been shown for

the initial thrombolysis, and now also for the avoidance of late postthrombotic changes *("Streptokinase or Heparin in the Treatment of Deep Vein Thrombosis. Follow-up Results of a Prospective Study". Acta Med Scand 1982; 211: 65).*

In the meantime we also conducted a RCT with the same principles in patients with major pulmonary embolism *("A controlled clinical trial of streptokinase and heparin in the treatment of major pulmonary embolism" Acta Med Scand 1978; 203: 465).* Altogether 25 patients with major pulmonary embolism were treated with streptokinase (n=14) or heparin (n=11) for 72 hours before angiographic control. The angiographic evidence of thrombolysis was significantly greater ($p<0.01$) in the streptokinase group. However, these results did not influence mortality which was low (2 in the heparin group, 1 in the streptokinase group, 2 of them after 3-4 weeks because of carcinomas), and bleeding was not a serious problem in any patient. It was concluded that patients with life-threatening pulmonary embolism should be offered the benefit of streptokinase.

Back to RCT in cardiovascular disease

Again focusing on the evolving intracoronary thrombus in the initial phase of myocardial infarction, I was happy to treat for the first time in Scandinavia a young man (39 years old) with impending infarction with direct intracoronary streptokinase. We could visualize the ongoing thrombolysis in the cath lab, and the clinical successful recovery of the patient. The case report was published in Scand J Haematol in a special issue in honour of my scientific mentor professor Hans Christian Godal (*"Successful treatment with selective intracoronary streptokinase in impending myocardial infarction", Scand J Haematol 1983, Suppl.39; 39.).* As a curiosity, I like to mention that in 2008, 27 years later, I received a message from his wife that the patient was still active in life at an age of 66 to great pleasure for his family, and she still remembered the exciting day in 1981.

To further focus on the long-term secondary prevention with anticoagulation after myocardial infarction, we started the prospective, randomized, placebo-controlled **WARIS trial** (The WArfarin ReInfarction Study), with later professor MD Pål Smith as the PhD candidate, and the later professor Ingebjørg Seljeflot as responsible for the laboratory services, all undertaken at the Red Cross Clinic in Oslo. Altogether 1214 patients with acute myocardial infarction from hospitals in the Oslo region were included and followed for 3 years for clinical end-points. At the end of the treatment period there had been 123 deaths in the placebo group and 94 in the warfarin group, a reduction in risk of 24% ($p=0.027$). Further, reinfarctions were noted in 124 in the placebo group and in 82 in the warfarin group, a reduction of 34% ($p=0.0007$). Of special note, a reduction in cerebrovascular accidents of 55% in the warfarin group as compared with the placebo group was observed (20 vs. 44) ($p=0.0015$). It was concluded that long-term therapy with warfarin has an important beneficial effect after myocardial infarction and can be recommended in the

treatment of patients who survive the acute phase *("The effect of warfarin on mortality and reinfarction after myocardial infarction." N Engl J Med 1990; 323: 147).* In light of the pretty large number of patients in the WARIS trial, per protocol subgroup analysis of possible differences in prospectively important subgroups was undertaken. It appeared that in addition to high age, the presence of diabetes seemed to induce reduced effect of warfarin on both reinfarction and mortality *("Effects of long-term anticoagulant therapy in subgroups after myocardial infarction." Arch Int Med 1992; 152: 993).*

While still working at the Red Cross Clinic and continuously being interested in RCTs we started out with the SHunt Occlusion Trial **(SHOT**) in close cooperation with the heart surgeons at Ullevål University Hospital. The main focus was on the reported pretty high occlusion rate of venous coronary by-pass grafts, thought to partly be caused by atherothrombosis. At the same time our interest was also focused on the possible positive role of very long chain marine omega-3 fatty acids on atherothrombosis. So our collegue, specialist in cardiology and working in Department of Cardiology Jan Eritsland was recruited as the PhD candidate. Again with Ingebjørg Seljeflot as responsible for blood sampling and biobanking for substudies. Altogether 610 patients undergoing aorto-coronary by-pass grafting were recruited to this randomized, controlled study with additional stratification to either aspirin or warfarin as anticoagulants. The patients were followed every 3 months until repeated coronary angiography after 1 year. Objective angiographic evaluation showed vein graft occlusion rates per distal anastomosis in 27% in the fish oil group and 33% in the control group (OR 0.77, p=0.034). When looking into the relative change in serum phospholipid n-3 fatty acids during the study period, significantly fewer patients with vein graft occlusions were found with increasing levels of n-3 fatty acids (p for trend=0.0037). As a fact the number of patients with ≥1 vein graft occlusion in the upper quartile of change was only half of that in the lowest quartile. No significant differences were noted between the stratified groups of aspirin and warfarin. It was concluded that there was a positive association between the serum levels of n-3 fatty acids and vein graft patency, supporting the notion that an increased dietary intake of n-3 fatty acids may confer protection against atherothrombosis *("Effects of Dietary Supplementation With n-3 Fatty Acids on Coronary Artery Bypass Graft Patency", Am J Cardiol 1996; 77: 31).*

With a trip back to venous thrombo-embolism we were introduced to the occurrence of postoperative thromboemboli after hip replacement surgery, previously reported to be as high as 60% during hospital stay, and even higher later postoperatively. As an orthopedic surgeon dr. Ola E. Dahl introduced a prospective, randomized, placebo-controlled study with the anticoagulant Dalteparin (Low-molecular weight heparin,Fragmin) for 5 weeks postoperatively *("Prolonged Thromboprophylaxis Following Hip Replacement Surgery - Results of a Double-blind, Prospective, Randomised, Placebo-controlled Study with Dalteparin (Fragmin)", the "PTP study", Thromb Haemost 1997; 77: 26).*

Altogether 308 patients were included in the **PTP study**. It should be mentioned that more than 80% had their prosthesis fixed with bone cement. In the first 7 postoperative days all patients received Fragmin, Dextran and below-knee graded elastic stockings. On day 7 randomisation to double-blindly injection of Dalteparin 5000 IU or placebo (sodium chloride) as a single daily injection, was performed. Venography on day 7 visualized proximal DVT in 41 patients, and later 28 patients were excluded from the final efficacy evaluation for practical reasons, evenly distributed in the randomised groups. 227 patients underwent final ITT analyses with venography, lung scan and chest x-ray after 35 days. Angiographic DVT was found in 22 out of 114 (19.3%) in the Dalteparin group, compared with 33 of 104 (31.7%) in the placebo group (RR 0.61; 95% CI, 0.38-0.97; p=0.034). High and intermediate probability pulmonary embolism (PE) on day 35 was found in 4 of 111 (3.6%) patients in the Dalteparin group and in 6 of 105 (5.7%) patients in the placebo group. It was concluded that the occurrence of DVT increased significantly from 1 to 5 weeks after hip replacement surgery in patients without prolonged thromboprophylaxis. One daily self-administered dose of Dalteparin, 5000 IU, significantly counteracted the progression of DVT. In addition, development of serious PE was prevented and a thrombolytic tendency was observed in patients who continued Dalteparin prophylaxis for 5 weeks. On this basis, we felt it reasonable to recommend prolonged thromboprophylaxis for 5 weeks after hip replacement surgery. Several substudies, mainly focusing on the use of prosthesis fixation with cement, showing prothrombotic effects, were performed.

Inspired by the background for the SHOT study and the possible effect of very long chain marine omega-3 fatty acids on atherothrombosis, we turned our focus towards the reports of restenosis after initially successful percutaneous transluminal coronary angioplasty (PTCA), the mostly used invasive method for elective myocardial revascularization in these days. Being back at Ullevål University Hospital as a professor, and also with Ingebjørg Seljeflot as a Research Medical Technologist, and with MD Odd Johansen as a PhD candidate we launched the Coronary Angioplasty Restenosis Trial (**CART)**.
Altogether 500 patients accepted for elective PTCA were included and randomized to receive either 5.1g/d of n-3 PUFA or corn oil as placebo, starting two weeks prior to the PTCA and followed for six months until restenosis evaluation by quantitative angiography. The evaluation showed that one or more restenoses occurred in 90/196 (45.9%) in the n-3 PUFA group and in 86/192 (44.8%) in the placebo group (OR 1.05, 95CI 0.69-1.59, p=0.82). It was concluded that supplementation with 5.1g n-3 PUFA/day for six months, initiated at least two weeks prior to coronary angioplasty did not reduce the incidence of restenosis *(" n-3 fatty acids do not prevent restenosis after coronary angioplasty. J Am Coll Cardiol 1999; 33: 1619).* It should be stressed that the dose of n-3 PUFA in CART was very high, and was chosen because some data in the literature pointed to a "higher the better" for supplementation with n-3 PUFA when CART was started.

In a follow-up study, called the "**post-CART study**" we looked into possible mechanisms for the "neutral" results of the CART. Fifty-four of the patients from the CART study were randomly invited into a follow-up study, 23 from the n-3 PUFA group and 31 from the placebo group. Both groups were given 5.1g n-3 PUFA/day for another 4 weeks, and circulating levels of a series of markers of endothelial function (tissue plasminogen activator antigen; von Willebrand factor; thrombomodulin, P-selectin; E-selectin; VCAM-1), in addition to markers of lipid peroxidation (vitamin E; TBARS) were measured before and after these 4 weeks. Differences between the groups were found before the 4 weeks, as the original n-3 PUFA group had lower von Willebrand factor and thrombomodulin, but higher levels of E-selectin and VCAM-1. After 4 weeks these differences disappeared, whereas vitamin E decreased and TBARS increased in the initial placebo group. It was concluded that n-3 PUFA supplementation in this group of patients may decrease haemostatic markers of atherosclerosis, whereas markers of inflammation are increased. The latter may be the result of lipid peroxidation *("The effect of supplementation with omega-3 fatty acids on soluble markers of endothelial function in patients with coronary heart disease. Arterioscler Thromb Vasc Biol 1999; 19: 1681).*

Looking back to the positive results of the WARIS trial (vide supra), we were often asked whether aspirin could match warfarin in the secondary prophylaxis after myocardial infarction, being aware of the probable role of platelets in the generation of coronary thrombi. Therefore, we launched the **WARIS-II trial** ("*Warfarin, aspirin, or both after myocardial infarction". New Engl J Med 2002; 347: 969). It* was a national, randomized, multicenter trial of 3.630 patients in Norway with the study center at Ullevål University Hospital with MD Mette Hurlen as the coordinating candidate. Altogether 1216 patients received warfarin (in a dose intended to achieve an INR of 2.8-4.2), 1206 patients received aspirin (160mg daily), and 1208 patients received aspirin (75mg daily) combined with warfarin (in a dose intended to achieve an INR of 2.0-2.5). The mean duration of observation was 4 years. The primary outcome, a composite of death, nonfatal reinfarction, or thromboembolic cerebral stroke, occurred in 241 of 1206 patients receiving aspirin (20.0%), 203 of 1216 receiving warfarin (16.7%; rate ratio compared with aspirin, 0.81; 95%CI 0.69-0.95; p=0.03), and 181 of 1208, receiving warfarin and aspirin (15.0%; rate ratio as compared with aspirin, 0.71; 95%CI 0.60-0.83; p=0.001). Episodes of major, non-fatal bleeding were observed in 0.62% of patients per treatment-year in both groups receiving warfarin and in 0.17% of patients receiving aspirin (p<0.001). It was concluded that warfarin, in combination with aspirin or given alone, was superior to aspirin alone in reducing the incidence of composite events after an acute myocardial infarction, but was associated with a higher risk of bleeding.

Being still interested in the possible effects of n-3 fatty acids on atherosclerotic heart disease, we now turned to the possibility that differently fed farmed salmon might contain different amounts of relevant fatty acids in their fillets, probably being transferred to human patients after ingestion. By contact with the veterinarian Sverre Ludvig Seierstad and the Norwegian School of Veterinary Science, we launched the so-called "**Fiord to Table**" (Fjord til Bord) trial. Sverre Ludvig was responsible for the production of farmed salmon in a center at the West coast of Norway, and collaborations with the supplier of feeding pellets (Nutreco, Stavanger), as well as the National Institute of Nutrition and Seafood Research in Bergen were established. In a double-blinded intervention study, 60 patients with documented coronary heart disease were randomly allocated to three groups consuming approximately 700 g/week for six weeks of differently fed Atlantic salmon: 100% fish oil (FO), 100% rapeseed oil (RO) or 50% of each (FO/RO), resulting in fillets with high, low and intermediate levels of marine n-3 PUFAs. Patient analyses before and after the intervention period included serum fatty acid profile, serum lipoproteins, and markers of vascular inflammation. We found that the serum fatty acid profiles of the patients after the intervention period mirrored those of the corresponding salmon fillets and the respective salmon feeds. Significant differences between the groups were obtained, especially for the levels of total n-3 PUFAs and the n-3/n-6 ratio, which were markedly increased in the FO group in contrast to the two other groups ($p<0.02$ for all). Additionally, significant reductions of serum triglycerides and of vascular cell adhesion molecule-1 and interleukin-6 were obtained in patients receiving the FO diet when compared with the two other groups ($p< 0.05$ for all). It was concluded that tailor-made Atlantic salmon fillets very high in n-3 PUFAs of marine origin seem to impose favourable biochemical changes in patients with CHD when compared with ingestion of fillets with intermediate and low levels of marine n-3 PUFAs, when replaced by rapeseed oil *("Dietary intake of differently fed salmon; the influence on markers of human atherosclerosis". Eur J Clin Invest 2005; 35: 52).*

With continuous interest in dietary intake and supplementation of very long chain marine n-3 fatty acids on individuals of risk for atherosclerotic heart disease, we launched the randomized so-called **DOIT study** (Diet and Omega-3 Intervention Trial) with MD Elsa M Hjerkinn as the PhD candidate. The patients population was recruited in survivors of the previous Oslo study (Lancet 1981) in patients with hypercholesterolemia, originally comprising 1232 men at high risk of CVD. Altogether, 563 elderly men were randomized into 4 groups: Dietary counselling according to European guidelines (Mediterranean-like) and placebo; n-3 PUFA supplementation (2.4 g/day of Pikasol, Lube, Denmark), no dietary counselling; dietary counselling and n-3 PUFA supplementation; control (no dietary conselling and placebo). Patients were followed for 3 years before evaluation for progression of atherosclerosis with carotid plaques, carotid intima media thickness and by pulse-wave propagation (PWP). In addition, samples for serum lipids and fatty acids were gathered and preserved in biobanks. The following results were observed: In the diet only group, the

carotid intima media thickness (CIMT) increase was significantly less than in the control group (p=0.018). Significant increase in carotid plaques score and plaques area were observed in all 4 groups, but without between group differences. Changes in CIMT and HDL-cholesterol were negatively correlated (adjusted p<0.001). PWP time decreased significantly in the control group (p=0.002), reflecting reduced arterial elasticity. In the group receiving n-3 PUFA only, PWP time increased significantly compared with the control group (p=0.013). We concluded that reduced CIMT was observed after dietary counselling, whereas n-3 PUFA supplementation imposed a favourable effect on arterial elasticity *("Effect of diet and/or very long chain n-3 fatty acids on progression of atherosclerosis, evaluated by carotid plaques, intima media thickness and by pulse wave propagation in elderly men with hypercholesterolemia". Eur J Cardiovasc Prev Rehab 2006; 13: 325).*
Later, a series of substudies have been undertaken on samples from the extensive biobank, mainly focusing on the possible effects in the randomised groups on components of the metabolic syndrome, as well as on markers of inflammation and atherosclerosis, resulting in about 25 additional publications, some of them as part of the PhD thesis of later professor Marius Trøseid in 2010, as well as in the PhD thesis of later professor Thomas Weiss in 2012.

Now turning our interest into patients with atrial fibrillation (AF), we launched the **CAPRAF study** *("CAndesartan in the Prevention of Relapsing Atrial Fibrillation". Int J Cardiol 2007; 120: 85").* Based on studies reporting preventing effects on the incidence of AF with angiotensin II type 1 receptor blockers (ARBs), altogether 171 patients with persistent AF were randomized into a double blind, placebo-controlled study to receive 8mg/day of candesartan or matching placebo for 3-6 weeks before cardioversion. Successfully cardioverted patients received 16mg/day or matching placebo from the day after the cardioversion until follow-up after 6 months, or until relapse of AF was documented. A total of 68 patients in the candesartan group and 69 patients in the placebo group were successfully cardioverted. Forty-eight patients (71%) in the candesartan group and 45 (65%) in the placebo group had a recurrence of AF during 6 months follow-up. Median time to recurrence was 8 and 9 days in the candesartan and placebo groups, respectively. The differences between the groups were not statistically significant. We concluded that treatment with the ARB candesartan for 3-6 weeks before and 6 months after electrical cardioversion (ECV) had no effect on the recurrence rate of AF. Although the CAPRAF study was "neutral", we developed a large biobank from the patients allowing a series of interesting studies on possible markers of maintenance of sinus rhythm after ECV for AF. Of positive results to be mentioned are that low serum levels of the inflammatory markers CRP and E-selectin at baseline were associated with maintenance of sinus rhythm after ECV *(Am J Cardiol 2007; 99: 1544).* An additional original observation from the biobank, linking the haemostatic system to AF was that baseline levels of the fibrinolytic inhibitor, plasminogen activator inhibitor type 1 (PAI-1) was highly associated with increased risk of AF recurrence. In parallel, a similar highly significant association was observed with body mass index (BMI) and risk of AF recurrence. It was discussed that the active fibrinolytic enzyme, plasmin might

also be involved in positive remodelling of the myocardium, possibly being inhibited by PAI-1. As it is well known that PAI-1 is actively produced in adipose tissue, the observations might be discussed together *(Thromb Res 2008; 121: 447).*
The latter observations fit elegantly into our scope of translational research. In 2008 later professor Arnljot Tveit defended his PhD thesis based on the CAPRAF studies.

In the same time frame as the CAPRAF study, we were engaged with the upcoming issue of potential curative effect of autologous stem-cells in patients with acute myocardial infarction. In close collaboration with the cardiologists at Rikshospitalet, we launched the **ASTAMI** (Autologous Stem-Cell Transplantation in Acute Myocardial Infarction) study. Altogether 100 patients with acute anterior wall myocardial infarction subjected to PCI with stent implantation of culprit lesion in the left anterior descending coronary artery, were randomized to injection of autologous mononuclear bone marrow cells (BMC) or control, 50 patients in each group. MD Svein Solheim was the PhD candidate in CCHR, Ullevål and MD Ketil Lunde the corresponding PhD candidate at Rikshospitalet. Baseline recordings of "Single-photon-emission computed tomography" (SPECT) and echocardiography were undertaken on days 4-7 after the AMI before the bone marrow aspiration and intracoronary mononuclear BMC injection in the actively treated group. Magnetic resonance imaging (MRI) was undertaken 2-3 weeks after the MI, and SPECT, echocardiography, MRI, and coronary angiography were repeated 6 months after the MI. The main results in the ASTAMI study were neutral, that is with no significant difference between the randomized groups on global left ventricular function with the methods used. The scientific report *"No effects on global left ventricular function and infarct size of intracoronary injection of autologous bone marrow mononuclear cells in acute myocardial infraction" was published in N Engl J Med 2006; 355: 1199.* The study was included in the PhD theses of both candidates. Later, a series of additional "translational" studies based on the extensive biobank from the ASTAMI study have been published, mainly on the effects of intracoronary injection of autologous bone marrow cells on inflammation.

Being continuously interested in thrombolysis, also as a "first line treatment" in patients with ST-elevation acute myocardial infarction (STEMI), routinely administered during ambulance transport to Ullevål university hospital when more than 90 minutes transfer delay until PCI was expected for patients from rural areas. An open question was if immediate transport to the PCI unit was favourable when compared to standard treatment at the local hospitals, with early transfer only if indicated for rescue or clinical deterioration. To try to answer this question, the **NORDISTEMI** (NORwegian study on DIstrict treatment of ST-Elevation Myocardial Infarction) trial was launched. With MD Ellen Bøhmer, working partly at Lillehammer hospital, as the PhD candidate, and later professor Sigrun Halvorsen as the primary supervisor, altogether 266 patients with acute STEMI living in rural areas with more than 90 minutes transfer delays to PCI were included. They were treated with

tenecteplase, aspirin, enoxaparin and clopidogrel in the ambulance, and randomized to immediate transfer for PCI or to standard management in the local hospitals, with early transfer only as mentioned. The primary outcome was a composite of death, reinfarction, stroke, or new ischemia at 12 months, analysed by "intention-to treat". The primary end point was reached in 28 patients (21%) in the early invasive group compared to 36 patients (27%) in the conservative group (HR 0.72, 95%CI 0.44-1.18, p=0.19). However, the composite of death, reinfarction and stroke was significantly reduced in the early invasive group (6% vs. 16%, HR 0.36, 95%CI 0.16-0.81, p=0.01). It was concluded that immediate transfer for PCI did not improve the primary outcome significantly, but reduced the rate of death, reinfarction, or stroke at 12 months in patients with STEMI, treated with thrombolysis and clopidogrel in areas with long transfer distances *("Efficacy and Safety of Immediate Angioplasty Versus Ischemia-Guided Management After Thrombolysis in Acute Myocardial Infarction in Areas With Very Long Transfer Distances". J Am Coll Cardiol 2010; 55: 102.).*

Continously being interested in haemostasis, and turning also to the effects of platelets, the question of possible "resistance" to the effects of aspirin was becoming "hot", mainly in relation to the recommended use of this drug in patients with coronary heart disease with intracoronary thrombus formation as a main pathogenetic factor for myocardial infarction. With MD Alf-Åge Reistad Pettersen as the PhD candidate the **ASCET study** (ASpirin non-responsiveness and Clopidogrel Endpoint Trial) was launched. Altogether, 1001 patients of both sexes, 18-80 years old with angiographically documented coronary artery disease (CAD), and on long-term single antiplatelet therapy with aspirin (160mg/d) were included. After laboratory testing for Platelet reactivity while on aspirin, patients were randomized to continue with aspirin 160mg/d or be switched to clopidogrel 75mg/d, and followed for 2 years for clinical end-points composed of death, myocardial infarction, ischemic stroke and unstable angina. High on-aspirin residual platelet reactivity (RPR) was determined by the PFA 100 platelet function analyzer system. After 2 years, 106 primary end-points were registered. The prevalence of high RPR was 25.9%. The prevalence of high RPR did not significantly influence the primary end-point in the aspirin group (13.3% vs. 9.9%, p=0.31). The composite end-point rate in patients with high on-aspirin RPR treated with clopidogrel was not significantly different from that of patients treated with aspirin (7.6% vs. 13.3%, p=0.16). It was concluded that in stable, aspirin-treated patients with CAD, high on-aspirin RPR did not relate to clinical outcome, and did not identify a group responsive to clopidogrel. *("High On-Aspirin Platelet Reactivity and Clinical Outcome in Patients With Stable Coronary Artery Disease: Results from ASCET". J Am Heart Ass 2012; doi:10.1161/JAHA.112.000703).* Along with the ASCET study a large biobank was generated, and more than 20 translational studies have been published from this population with 1001 CAD patients, most of them as part of later PhD theses.

When MD Rune Byrkjeland contacted us in CCHR in 2010 with his interest in physical training and devoted for clinical research, we launched the **EXCADI study** (EXercise training in patients with Coronary Artery disease and type 2 Diabetes). At that time physical training was documented beneficial for patients with coronary artery disease (CAD) or diabetes type 2 (DM2), however with few data on the combination of the two, which was regularly seen. Thus, 137 patients were included, with the last follow-up in March 2013. CAD was verified with coronary angiography in all patients before randomization into either the exercise group or the control group. The exercise group underwent a 12-month combined aerobic and resistance training program planned and conducted in collaboration with the Norwegian School of Sports Sciences, and the control group continued with normal follow-up by their general practitioner. The primary outcomes were HbA1c and VO2peak. No differences in changes between the randomized groups were observed in HbA1c and VO2peak, whereas ventilator threshold and time to exhaustion increased significantly in the exercise group compared with the controls. Furthermore, in patients without previous acute myocardial infarction and diabetic microvascular complications (n=46), the exercise group did improve HbA1c and VO2peak compared with the controls. It was concluded that no significant effects of exercise training on HbA1c or VO2peak were observed in patients with CAD and DM2, although improvements were seen in patients without vascular complications beyond CAD, implying that the degree of vascular disease may influence exercise responses. Ventilatory threshold and time to exhaustion did increase significantly, indicating improved exercise performance despite the minor change in VO2peak *("Effects of exercise training on HbA1c and VO2peak in patients with type2 diabetes and coronary artery disease". Diabetes &Vascular Disease Research 2015; 12: 325).* Again, a series of "translational studies" were undertaken on an extensive biobank, including insulin levels and HOMA index, measures of endothelial activation and carotid intima-media thickness. Later on at least 15 sub-studies on the EXCADI biobank have been published from CCHR.

Returning to our interest in marine omega-3 fatty acids, we in 2012 launched our long-lasting randomized trial, the **OMEMI study** (Omega-3 fatty acids in Elderly patients with Myocardial Infarction). It was designed as a multicenter, placebo-controlled, double-blind clinical trial conducted by independent investigators at CCHR. The study was finalized in 2021 with the publication *"Effects of n-3 Fatty Acid Supplements in Elderly Patients After Myocardial Infarction. A Randomized, Controlled Trial", Circulation 2021; 143: 528,* after being presented as a "Late breaking trial" during the AHA congress in the US this year. During the study period 4 PhD candidates worked on the study, 2 at Akershus university hospital (AHUS) (Peder L. Myhre and Sjur H.Tveit) and 2 at CCHR, OUS, Ullevål (Kristian Laake and Are A.Kalstad). All of them have finalized their thesis, the last two in 2022.
Altogether 1027 patients, 70-82 years old with a recent acute myocardial infarction were randomized into the "N-3 group" receiving 1.8 g very-long-chain, marine n-3 PUFA (930mg eicosapentaenoic acid (EPA) and 660mg docosahexaenoic acid (DHA)) or the placebo group

(same amount of corn oil) daily in addition to standard of care for 2 years. The primary end-point was a composite of non-fatal AMI, unscheduled revascularization, stroke, all-cause death, and heart failure hospitalization. The secondary end-point was new onset atrial fibrillation. Follow-up data were available for 1014 patients who were included in the intention-to-treat analysis. All end-points were verified by an independent expert committee. The primary end-point occurred in 108 (21.4%) patients in the n-3 PUFA group versus 102 (20.0%) on placebo (hazard ratio 1.08 (95%CI 0.82-1.41, p=0.60). The secondary end-point occurred in 28 (7.2%) patients on n-3 PUFA versus 15 (4.0%) on placebo (1.84 (0.88-3.45, p=0.06). Median serum changes in EPA and DHA were +87% and +16% for n-3 PUFA versus -13% and -8% for placebo. Major bleeding occurred in 54 (10.7%) and 56 (11.0%) in the n-3 PUFA and placebo groups, respectively (p=0.87). Similar results were found in per-protocol analysis (n=893). It was concluded that we could not detect reduced incidence of cardiovascular events or all-cause death in our elderly patients with a recent AMI, treated with 1.8 g very-long-chain, marine n-3 PUFAs daily for 2 years. However, an increased incidence of new atrial fibrillation of borderline significance was noted in the n-3 PUFA group, deserving new research. As usual in CCHR a series of translational studies based on an extensive biobank have been undertaken and are ongoing, mainly related to ageing, telomere length and cardiac remodeling in relation to atrial fibrillation. Of special interest is the observation that new onset atrial fibrillation was found to be associated with the serum levels of EPA, with increased incidence in patients with the highest absolute serum levels and also with the largest EPA increase during the study period. These findings being "opposite" to a lower risk of incident AMI, coronary revascularization, stroke or hospitalization for heart failure with increased levels of serum EPA (*Recently published in J Int Med 2021; 00:1-11. DOI: 10.1111/joim.13442* with Peder L. Myhre as the first author).

As will emerge, I have had the pleasure to work with highly educated, devoted and positive persons through this "scientific journey", from the beginning in the milieu created by professor Hans Christian Godal at Haematological Research laboratory, Ullevål University Hospital, with outstanding colleagues who also became my friends. Further to the coincident meeting with the excellent Medical Technologist Ingebjørg Seljeflot, being Head of the laboratory at Red Cross Clinic, where I worked for 8 years. She was obviously fascinated by research and later (1991) moved to Ullevål hospital as a laboratory research leader. Together we established a "labo-clinical symbiosis", where I came up with "unsolved problems" from the clinical work, taken further into translational thinking in the laboratory by Ingebjørg, specially interested in evolving methods for further understanding of mechanisms of disease states and their treatment. She also conducted her own research and defended her PhD thesis in February 1997, 25 years ago. ("Studies On The Fibrinolytic System, with special reference to Laboratory Methods for evaluation of Global Fibrinolysis, and to some Regulatory Mechanisms of t-PA and PAI-1"). After further continuing her own research, and additionally supervising new PhD candidates, she was elected a professor in "Cardiovascular

translational research" at University of Oslo in 2008. Together we established Center for Clinical Heart Research (CCHR), where about 45 PhD candidates have been educated. Our simple slogan: "Team building for individual excellence" has shown fruitful (picture vide infra). In 2007 we were "discovered" by the philanthrop and "moneymaker" Stein Erik Hagen who through his CANICA company has supported us with NOK 5 mill/year, enabling us to "look ahead" and establish international contacts as well as start scientific projects for young devoted persons, working for Excellence and PhD degrees.

Based on the achieved results in the CCHR I was in 2005 honoured by "Hjerteprisen" from Norwegian Health Association, delivered by our King Harald V in the Aula of Oslo University (picture vide infra), and in 2017 by being created "Doctor Medicinae honoris Causa" ("Æresdoktor") at Aalborg University, Denmark.

Thank you all for inspiring company and friendship during this almost 55 years long fascinating scientific journey with randomized clinical trials and penetrating translational research, looking into the secrets of human biology. It has been a real privilege to have had this opportunity until my age of 83 years, when I now retire as a professor emeritus with the greatest satisfaction, hoping for the positive future for the CCHR.

Oslo, September 2022.

Harald Arnesen

From the Norwegian Health Association's "Hjertepris" 2005

CCHR' Scientific Symposium Norefjell 2019