



Influence of acute alcohol intoxication on certain immune reactions

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Abstract

Background Long-term alcohol abuse is a potent immunomodulator, and alcohol abusers have increased risk of bacterial infections after surgery. In experiments, acute alcohol intoxication suppresses certain immune reactions and may co-act with trauma to increase risk of post-trauma infectious complications. The aim was to evaluate immune reactions during, an evening of social drinking.

Methods We studied 13 healthy, non-smoking volunteers having red wine (1 g of ethanol/kg body weight) with a three course dinner. Delayed type hypersensitivity (DTH) skin test, plasma plasminogen activator inhibitor-1 (PAI-1), plasma myeloperoxidase (MPO) and plasma histamine, representing parts of the immune response with specific relation to the host defence against bacterial antigens, were tested two weeks before the dinner, immediately prior to the dinner, when the blood alcohol level peaked, and one week after the dinner. The volunteers abstained from alcohol in the two weeks leading up to the dinner and one week afterwards.

Results The median peak blood alcohol level reached 92 (51-124) mg/dl, equivalent to 20 (11-27) mmol/l. No change in the DTH response was found, $p = 0.76$. PAI-1 concentration in plasma increased significantly from 3 (1-13) ng/ml just before dinner to 20 (5-75) ng/ml at the time of alcohol peak level, $p < 0.01$. Similarly, plasma histamine increased from 5.8 (4.7-10) nmol/l to 7.1 (5.6-10) nmol/l, $p < 0.01$ and plasma MPO decreased from 96 (42-158) to 91 (41-125) ng/ml, $p < 0.05$. After one week of abstinence, PAI-1, histamine and MPO returned to pre-dinner level.

Conclusion The study showed significant changes in various parts of the host's defence to bacteria following acute alcohol intoxication. Potentially, this may be detrimental for patients, who have been traumatised at injury or surgery during alcohol intoxication, by contributing to enhanced susceptibility to post-trauma bacterial infectious complications.

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Introduction

Alcohol abuse leads to impaired immune response (1), and long-term alcohol abusers have increased risk of developing infections and neoplastic diseases (2;3).

Furthermore, acute alcohol intoxication has been found to be immunomodulatory in animal experiments. The cell-mediated immune response seems to react to alcohol in a dose dependent manner, and so low doses of ethanol may stimulate the immune response temporarily, while high doses may reduce the cellular immunity (4;5). In addition, acute ethanol intoxication is known to inhibit neutrophil delivery to sites of inflammation, reduce adherence of neutrophils to endothelial cell surfaces, transiently suppress chemotactic function and reduce the bactericidal ability to kill *Staphylococcus aureus*, when doses of 3 g/kg of ethanol or higher are administered experimentally (6;7). Similar concentrations of ethanol (25-100 mM) induces monocyte and

macrophage synthesis of interleukin-10 (IL-10) and impairs tumor necrosis factor (TNF- α) synthesis, which may contribute to an early inhibition of inflammatory response to bacterial stimuli (8;9). This appears to inhibit the ability to clear bacteria and predisposes to infections in animals (10).

However, the knowledge of the effect of acute alcohol intoxication on various parts of the immune system in humans is sparse. Therefore, we studied the influence of social drinking and dining on parts of the immune response with particular emphasis on the bacterial immune defence (Table 1).

Materials and Methods

After signing an informed consent, 13 (8 men, 5 women) non-smoking, non-alcohol abusing healthy volunteers abstained from alcohol for two weeks leading up to and one week after the scheduled drink-



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Table 1 Blood components and immune analyses used to measure effect

Effects on	Measured by
Neutrophils	Myeloperoxidase, Interleukin-6
Basophils	Histamine
Monocytes	Interleukin-6, Myeloperoxidase
Platelets	Plasminogen activator inhibitor-1
T-lymphocytes	Delayed type hypersensitivity, skin test

ing event. Median age was 28 (range 18-65) years and body mass index was 23 (20-29) kg/m². None of the volunteers used medication and all were without a history or clinical signs of illness. Their usual alcohol intake was 1 (0-1) drink per day.

The study was approved by the Scientific Ethical Committee of Copenhagen (no. 01-401/96).

Alcohol Administration and Dinner

After two weeks of complete abstinence ensured by 800 mg disulfiram at inclusion, the volunteers consumed between 650 and 1000 ml of red wine (Spanish, 12.5 %) corresponding to 1.0 g of ethanol per kg body weight (i.e. 5.3 to 8.1 standard drink of 12 g ethanol) together with a three course dinner during a two hour study period. The dinner was composed of salmon, chicken with rice, and chocolate cake. Non-alcoholic beverages were available during the study period without any volume restrictions. The "happy evening" was followed by another week of abstinence from alcohol.

Blood Alcohol Level (BAL Determination)

Just before the study period, serum ethanol was analysed using the enzymatic method (Vitros, Johnson and Johnson, NY, USA). After finishing the alcohol drinking, BAL was monitored every ten minutes using the breath analysis (Lion Alcometer, Palmenco A/S, Denmark) and when BAL peaked, serum ethanol was determined again.

Blood Sampling

Blood samples were collected from a cubital vein at inclusion and analysed for B-leucocytes (Technicon, H3, Bayer, NY, USA), S-creatinine, S-albumin, S-transferrin, S-bilirubin, S-alkaline phosphatase, S-ALAT and S-amylase (Vitros, Johnson and Johnson, NY, USA). Blood was drawn before the study, immediately after reaching peak BAL, and after one week of abstinence, and put into ice-chilled endotoxin-free tubes (Becton-Dickinson, Mountain View, CA, USA) containing 0.5 ml sodium citrate (0.129 mol/l), and subsequently the blood was centrifuged at 4°C at 1200 G/10 minutes. The plasma was carefully separated from the cells, leaving at least 1 ml plasma on the top of the cell pellet and frozen at -70°C until analysed.

The following analyses were performed in duplicate using commercially available kits. The concentration of histamine was analysed by ELISA method (Immuno-technique SA, Marseilles, France); the detection limit was 0.5 nmol/l. PAI-1 was analysed by using a commercially available ELISA method (Monozyme, Hørsholm, Denmark) (11); the detection limit was 20 pg/ml. MPO by RIA (MPO RIA, Pharmacia & Upjohn AB, Uppsala, Sweden) with a detection limit of 8 ng/ml and IL-6 by ELISA (IL-6, Immuno-technique SA) with detection limit of 3.9 pg/ml.

Delayed Type Hypersensitivity (DTH)

DTH was measured by a skin test (Multitest®, Pasteur Mérioux SA, Lyon, France) that consisted of a plastic device capable of simultaneous application of a negative control of glycerine-saline diluent and seven DTH antigens: tetanus, diphtheria, streptococcus, tuberculin, candida, trichophyton, and proteus. The skin test was applied on the flexor surface of the forearm in the two week abstinence period, immediately before the dinner, and one week after. The cutaneous response was read 48 hours after the application and expressed as the sum of all indurated areas with a diameter of at least 2 mm.

Statistical Methods

The results are given in median and range values. The Wilcoxon Rank sum test was used for the paired data. The level of significance was $p \leq 0.05$.

Results

BAL measured by breath test was 0.0 in all volunteers before entering the study. During the drinking event, the peak level reached 16 (ranging 10-23) mmol/l. When BAL measured by breath test peaked, serum ethanol was 20 (11-27) mmol/l. B-leucocytes, S-creatinine, S-albumin, S-transferrin, S-bilirubin, S-alkaline phosphatase, S-ALAT and S-amylase were normal in all volunteers at study entry. During the "happy evening", the total leucocyte count increased significantly from 5.4 10⁹cells/l (4.7-10.5) to 6.6 (5.9-10.7), $p < 0.01$, due to a significant increase in total lymphocyte count from 2.3 10⁹cell/l (1.6-3.1) to 2.7 (1.9-3.5), $p < 0.01$. S-albumin and S-alkaline phosphatase were significantly increased, $p < 0.05$, while S-bilirubin decreased, $p < 0.01$. Granulocyte count, S-creatinine, S-transferrin, S-ALT, and S-amylase did not change.

The most marked difference between pre- and post-trial immune parameters was observed in platelet derived PAI-1 (Figure 1a). We also found a small but significant increase of plasma histamine (Figure 1b), and a small but significant decrease of MPO (Figure 1c). Both P-his-



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tamine and P-MPO returned to pre-study levels during the following week of abstinence.

Plasma IL-6 was undetectable in all volunteers throughout the study.

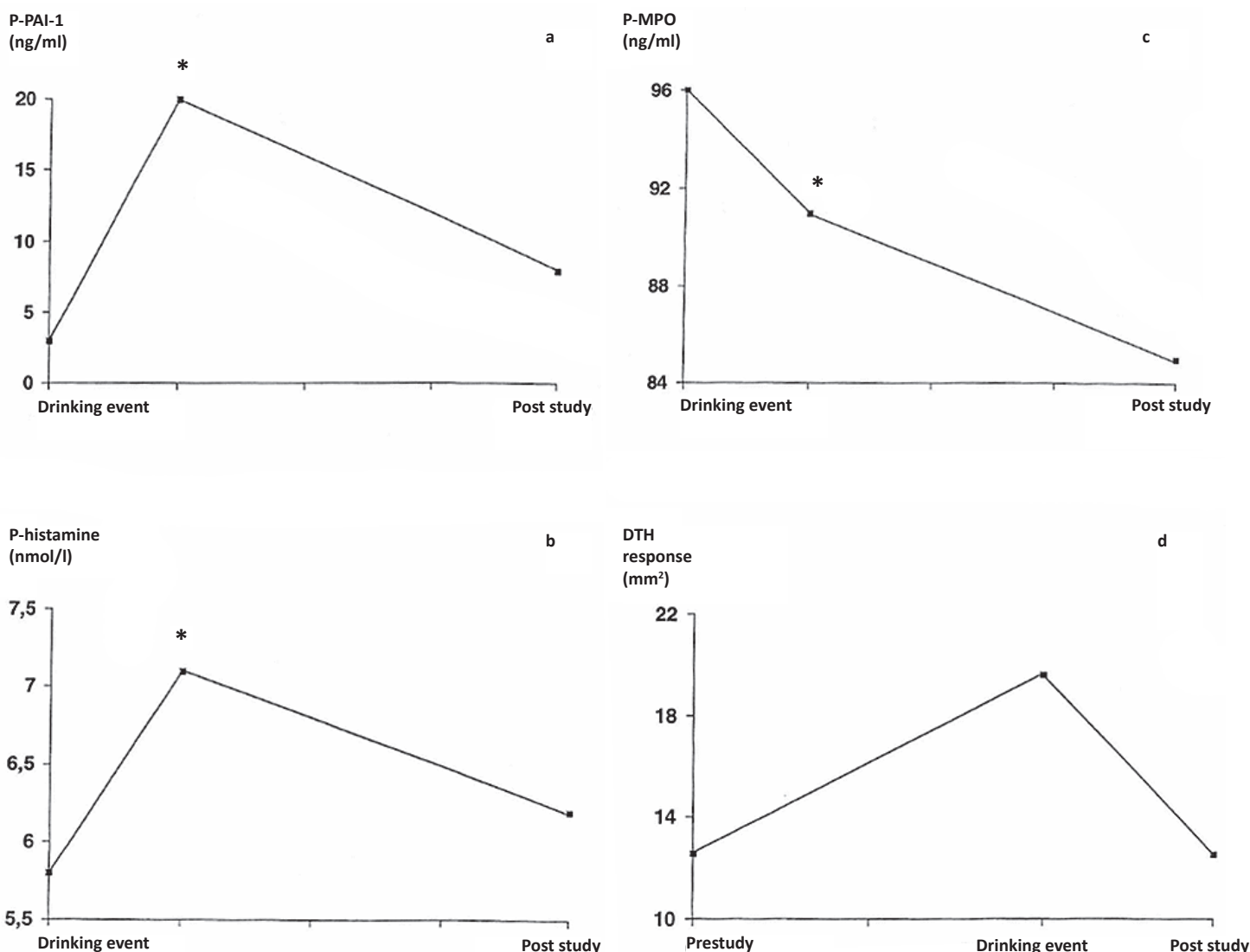
Delayed type hypersensitivity was not affected by the alcohol intoxication, $p = 0.76$ (Figure 1d).

Discussion

We found that the plasma concentrations of PAI-1, Myeloperoxidase and histamine increased significantly after acute alcohol intoxication. After one week of abstinence, the values returned to pre-dinner level.

Parts of the host defence have been investigated in relation to social drinking (8;12-16). Mandrekar et al evaluated the effect of a minor intake over 18 hours in humans, corresponding to less than one tenth of the intake in our study. They found attenuated monocyte inflammatory responses through inhibition of nuclear regulatory factor kappa B and induction of interleukin 10. Corberand et al. found, while examining neutrophil function, only a moderately and reversible depression of *S. aureus* phagocytosis by neutrophils (12). In a similar setup, Mohadjer et al. found small changes in cytokine levels, lymphocyte subpopulations, and mitogen stimulation (13). In another study, IL 2-induced lymphokine-activated killer activity was significantly reduced compared with activity before alcohol ingestion, but natural killer activ-

Figure 1 Immune alterations during drinking event and poststudy. a) Plasminogen activator inhibitor-1 (PAI-1). b) Histamine. c) Myeloperoxidase (MPO). d) Delayed type hypersensitivity (DTH). *: $p < 0.005$.





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ity was not affected. The alcohol amount ingested was small and BAL only reached 11-47 mg/dl (14). Veenstra et al. reported alteration in platelet function with a significant increase in plasma PAI-1 in the postprandial phase after drinking 30 g of alcohol (15). Increased plasma levels of PAI-1 have been found to impair fibrinolysis and potentially contribute to the formation of atherosclerosis and stroke (16;17). Both alcohol and food play a role in stimulating platelets to release PAI-1 (15;18;19). Minor intake of ethanol increases PAI-1 with about 10% (15), transient 20-50% time increase has reported after a carbohydrate rich meal (18;19), while a fat rich meal appears to be without influence (19). The sevenfold increase of PAI-1 in our study is probably a result of the higher alcohol ingestion, 1.0 g/kg bodyweight.

Histamine released from basophils and mast cells appears to participate in the regulation of the immune response. Thus, in physiological concentrations it may act as an immunostimulatory molecule (20), while increased concentrations may lead to immunosuppression (21). The small, significant increase of plasma histamine observed in our study is similar to the increase observed in patients with septicaemia (21) and in patients undergoing major surgery (22). Such concentrations may play a stimulatory role in inflammation (23) and may even impair neutrophil chemotaxis and T-lymphocyte proliferation in healthy volunteers (24). Furthermore, histamine in increased levels modulates various other pathologically processes (20).

Myeloperoxidase is an enzymes released by neutrophils when activated in immune reactions (25) and it may play a significant role in various pathophysiologically processes. Although significant, the decrease in plasma MPO observed at the peak alcohol level in our study is within the normal range observed in healthy, non-alcoholic volunteers (26). Therefore, the decrease may be normal variation, or it may be due to increased enzyme activity following increased levels of oxygen, free radicals induced by alcohol consumption (27).

We found no significant changes in cellular immunity and plasma IL-6, indicating that BAL normally reached in social drinking is too small to influence T-lymphocyte functions. In vitro experiments and animal studies confirm that a non-physiological level of serum ethanol has to be reached before a marked suppression of lymphocyte function can be found (4;5;28;29). Ethanol in physiological concentration seems to stimulate the immune system and improve host defence (29).

The elicited alterations of the immune defence and the consequences in a clinical situation when alcohol intoxi-

cation is followed by trauma are still not clear. Growing evidence showed that acute alcohol intoxication may co-act with trauma to increase post-trauma immunosuppression and risk of infection (30). Therefore, it still remains to be studied in more detail whether alcohol consumption in otherwise healthy people plays a significant role in enhancing the risk of complications for intoxicated traumatised patients. The small, but significant changes found in our healthy volunteers may eventually play a certain role, which potentially may be amplified with increased drinking. Further clinical studies are needed to clarify the effect of alcohol not only on healthy volunteers, but also on non-healthy people who are consuming alcohol.

Contribution Details

Conception and design: HT, NS,

Acquisition of data: HT, NS

Analysis and interpretation of data: HT, NS, KHJ, HJN

Drafting the article: HT

Revising the article critically for important intellectual content: HT, NS, KHJ, HJN

Final approval of the version to be published: HT, NS, KHJ, HJN

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Competing interest: None declared.

References

- (1) Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. *Alcohol Clin Exp Res* 2009; 33:220-2
- (2) Saitz R, Ghali WA, Moskowitz MA. The impact of alcohol-related diagnoses on pneumonia outcome. *Arch Int Med* 1997; 157:1446-52
- (3) Thygesen LC et al. Cancer Incidence among Patient with Alcohol Use Disorder – Long-Term Follow-Up. *Alcohol Alcohol* 2009; 44:387-91
- (4) Plackett TP, Kvacs EJ. Acute models of ethanol exposure to mice. *Methods Mol Biol*; 2008; 447:4-7
- (5) Dehne NE, Mendenhall CL, Roselle GA, Grossman CJ. Cell-mediated immune responses associated with short term alcohol intake: time course and dose dependency. *Alcohol Clin Exp Res* 1989; 13:201-5
- (6) MacGregor RR, Safford M, Shalit M. Effect of ethanol on functions required for the delivery of neutrophils to sites of inflammation. *J Infect Dis* 1988; 157: 682-9
- (7) Tamura DY, Moore EE, Patrick DA et al. Clinically relevant concentrations of ethanol attenuate primed neutrophil bactericidal activity. *J Trauma* 1998; 2:320-4
- (8) Mandrekar P, Catalano D, White B, Szabo G. Moderate alcohol intake in humans attenuates monocyte inflammatory responses: inhibition of nuclear regulatory factor kappa B and induction of interleukin 10. *Alcohol Clin Exp* 2006; 30: 135-9
- (9) Mandrekar P, Catalano D, Girouard L, Szabo G. Human monocyte IL-10 production is increased by acute ethanol treatment. *Cytokine* 1996; 8:567-77
- (10) Kawakami M, Meyer AA, Johnson MC, Rezvani AH. Immunologic consequences of acute ethanol ingestion in rats. *J Surg Res* 1989; 47:412-7
- (11) Grøndahl-Hansen J, Christensen IJ, Rosenquist C et al. High levels of urokinase-type plasminogen activator and its inhibitor PAI-1 in cytosolic extracts of breast carcinomas are associated with poor prognosis. *Cancer Res* 1993; 53:2513-21



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- (12) Corberand JX, Laharrague PF, Fillola G. Human neutrophils are not severely injured in conditions mimicking social drinking. *Alcohol Clin Exp Res* 1989; 13:542-6
- (13) Mohadjer C, Daniel V, Althof F, Maier H. Untersuchungen zum immunstatus nach einmaligem alkoholkonsum bei gesunden probanden. *DMW* 1995; 120: 1577-81
- (14) Bounds W, Betzing KW, Stewart RM, Holcombe RF. Social drinking and the immune response: impairment of lymphokine-activated killer activity. *Am J Med Sci* 1994; 307:391-5
- (15) Veenstra J, Kluff C, Ockhuizen T, Pol Hvd, Wedel M, Schaafsma G. Effects of moderate alcohol consumption on platelet function, tissue-type plasminogen activator and plasminogen activator inhibitor. *Thromb Haemost* 1990; 63:345-8
- (16) Mikhaelidis DP, Barradas MA, Jeremy JY. The effect of ethanol on platelet function and vascular prostanooids. *Alcohol* 1990; 7:171-80
- (17) Urano T, Kojima Y, Takahashi M et al. Impaired fibrinolysis in hypertension and obesity due to high plasminogen activator inhibitor-1 level in plasma. *Jpn J Physiol* 1993; 43:221-8
- (18) Madvescek M, Keber D, Stegnar M, Borovnicar A. Plasminogen activator inhibitor 1 response to a carbohydrate meal in obese subjects. *Fibrinolysis* 1990; 4, supp. 2:89-90
- (19) Keber D, Borovnicar A. Difference in insulin and plasminogen activator inhibitor (PAI) response after high carbohydrate as opposed to high fat meal. *Thromb Haemost* 1989; 62:291
- (20) Beer DJ, Rocklin RE. Histamine modulation of lymphocyte biology: membrane receptors, signal transduction, and functions. *Crit Rev Immunol* 1987; 7: 55-91
- (21) Nielsen HJ. Histamine-2 receptor antagonists as immunomodulators: New therapeutic views? *Ann Med* 1996; 28:107-13
- (22) Sitter H, Lorenz W, Klotter HJ et al. Elevated plasma histamine concentration as a sensitive real-time parameter for distinct phases of the surgical trauma: a tool for technology assessment. *Agents Actions* 1991; 33:203-7
- (23) Falus A, Meretey K. Histamine: an early messenger in inflammatory and immune reactions. *Immunol Today* 1992; 13:154-6
- (24) Bury TB, Corhay JL, Radermecker MF. Histamine-induced inhibition of neutrophil chemotaxis and T-lymphocyte proliferation in man. *Allergy* 1992; 47:624-9
- (25) Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 1997; 89:3503-21
- (26) Nielsen HJ, Skov F, Dybkjær et al. Leucocyte and platelet derived bioactive substances in stored blood: Effect of prestorage leucocyte filtration. *Eur J hematol* 1997; 58:273-8
- (27) Kurose I, Granger N. Evidence implicating xanthine oxidase and neutrophils in reperfusion-induced microvascular dysfunction. *Ann N Y Acad Sci* 1994; 723: 158-79
- (28) Szabo G. Monocyte-mediated immunodepression after acute ethanol exposure. *Adv Biosciences* 1993; 86:121-30
- (29) Mendenhall CL, Theus SA, Roselle GA, Grossman CJ, Rouster SD. Biphasic in vivo immune function after low-versus high dose alcohol consumption. *Alcohol* 1997; 14:255-60
- (30) Tønnesen H, Nielsen PR, Lauritzen JB, Møller AM. Smoking and alcohol intervention before surgery: evidence for best practice. *Br J Anaesth* 2009; 102:297-306

International Master in Health Promotion in Hospitals and Health Services - update

About the Master

The master is provided by WHO-CC for Evidence-based Health Promotion for Hospitals and Health Services in collaboration with a number of renowned Universities.

Courses are expected to begin in 2014.

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Due to a new European accreditation system for educational programmes, the development and thus the start-up of the proclaimed Master of Clinical Health Promotion has been delayed for a year.

As a result of the new accreditation system, the Master will be accredited in accordance with other university educational programmes and be worth a total of 60 ECTS point, which complies with the rules under the University Act.

WHO-CC for Evidence-based Health Promotion for Hospitals and Health Services (WHO-CC) are in dialogue with ACE Denmark, the Danish Accreditation Institute, who survey Danish applications on behalf of the European Committee. Also, WHO-CC has initiated a new partnership with University of Southern Denmark, who will take part in the devel-

opment as organising partner along with the existing partners; Lund University (Sweden), Charité - Universitätsmedizin Berlin (Germany), and University of Oslo (Norway).

Many of the International HPH Networks' member hospitals and health services have registered staff to participate in the development of the programme and with participation in the teaching activities. WHO-CC is pleased with the great support they have received from the International HPH Network and the many members.

