Cupric chloride crystallization with human blood
Study of pictures obtained in different pathologies

Marie-Thérèse Piva, Serge Lumbroso, Victor Sieso, Eric Monnin, Henri Mion, François Blanc, Bernard Magnan de Bornier

Summary:

We have studied the different pictures that appear after cupric chloride crystallization in the presence of blood in a bidimensional circular plane system. We describe the method in its various technical aspects and define the optimal conditions to obtain these pictures. In healthy subjects (chosen as controls), the crystallization was more regular than in non-healthy subjects. In 80 patients classified into 8 pathological groups there were crystallization alterations leading to geometrical figures. The different figures obtained are listed and schematized. An experimental procedure with less complex media than blood (i.e amino-acid solutions, serum albumin dilutions) demonstrates the influence of protein in eliciting forms. The statistical study showed significant differences between most of the patient groups and a relation between a single alteration »Form« frequency and an etiological group. Some crystallization patterns were also correlated with known variations of some different seric proteins. This report shows a one to one relationship between pathology and pattern allowing one to »visualize «a pathological state on a crystallographic picture.

Introduction

Experimented by the German biologist *E. Pfeiffer* (1931), the crystallization method was based on the study of patterns shown after water evaporation of a cupric chloride solution in the presence of complete blood, urine, or vegetal extract. In medicine, it has been deeply investigated by *A.* and *O. Selawry* (1957), and considered to be a good tool for orientation in diagnosis. This method was commented on and criticized by *E. Nickel* (1968). Since then, as far as we know, this technique, used for diagnostic purposes, has not shown any development according to the scientific literature. Nevertheless, *Tchebotareva et al.* (1990) used it in pediatry as a pyelonephritis diagnosis element. *Cocude et al.* (1992) evaluate this method for the medical follow up of pneumoconiosis of miners. *Shibata et al.* (1993) used it to probe a population suffering from senile dementia. We have assessed the validity of this technique in a hospital environment by confrontation with different organ pathologies. We studied the relationships between different pathological states and the crystallization forms, followed-up the variations of some seric proteins (pooled within a proteic profile) (*Giraudet et al.*, 1990) and we

examined their possible correlations with the modifications observed by crystallization. Finally, we tried to understand the formation of the different configurations by a rational approach including amino acid solutions or albumin dilutions. Our assays showed the importance of complex proteic mixture in the development of specific forms.

Material and methods

Patients: 35 healthy subjects, 17 men, 18 women, (average age : 48 years) were chosen as control group G0, defined below. 80 subjects in hospital were studied : 28 women and 52 men (average age : 61.5 years). They all originated from the Service de Medecine Interne E, CHU Montpellier, France. They were classified into 8 groups (G1 to G8) according to clinical, biological, anatomo-pathological and radiological data. So, we divided our sampling selection into nine groups as follows : G1, patients with alcoholic cirrhosis (n=25), G2, patients with liver carcinoma (n=12; 6 primary, 6 secondary), G3, patients with digestive tract illnesses (n=11; 3 malignant peritonitis, 3 adenocarcinomas, 5 gastric ulcers), G4, patients with pancreatic pathologies (n=11; 2 cancers, 9 non-insulino dependent diabetes mellitus), G5, patients with lung diseases (n=3; 2 cancers, 1 chronic abscess), G6, patients with cardio-vascular pathologies (n=6; 1 mitral deficiency, 3 auricular fibrillations, 2 hypertensions), G7, patients with renal diseases (n=7; 3 carcinomas, 4 renal failures), G8, breast pathologies (n=5; 2 cancers, 3 fibroadenomas), G0, 35 healthy subjects without any of the below mentioned pathologies and free from drugs for the previous four weeks before veinous puncture were referred as the control group.

Seric protein profile: It was composed of 9 proteins : immunoglobulins (IgM, IgG, IgA), fraction 3 of the complement (C3), and liver synthetized compounds : orosomucoïd (Oro), haptoglobin (Hpt), Transferrin (Trf), Albumin (Alb), and alpha-2-macroglobulin (A-2M) quantified by immunonephelometry (*Laurent et al.*, 1980) (Array-Protein-System-Beckmann). The usefulness of this protein profile relied on the relative variations of protein levels in the blood, as assessed in numerous pathologies (*Giraudet et al.*, 1990 ; *Meliconi et al.*, 1988 ; *Jayle et al.*, 1984 ; Frot et al., 1984). The results were expressed in g/l (grams per liter of serum) and in percentage of the median regarding control values for specific sex and age.

Crystallization: Standard proteic solutions : Glycine, Isoleucine, Histidine, Aspartic acid, Tyrosine, tryptophan, cystine, arginine were purchased from Merck (Germany). Human serumalbumin (HSA) was from Sigma. Solutions were performed in distilled water or in PBS buffer pH 7.4 at concentrations ranging 0.5-15 g/l. The operating procedure for amino acid solutions or HSA solutions is the same as for blood samples, i.e. 3 ml of HSA or amino acid dilution mixed with 5 ml of cupric chloride solution dropped into a glass cupel.

Blood samples were taken by venous punction. The blood samples collected in a dry glass tube (10 ml) were immediately poured and absorbed on an ash-free filter paper of middle porosity (Schleicher and Schull Germany). These blood paper spots,