New Clearance Method for Hepatologic Diagnostics. Clinical Study

Josef A. Tichý, Miloslav Loučka[1], Zdeněk M. Trefný, Markéta Hojerová, Josef Svačinka, Jindřich Müller[1], Michaela Friedrichova

Prague Institute of Chemical Technology, Department of Mathematics, Technická 5, 166 28, Prague 6 [1)]
Institute of Civilisation Diseases, Politických vězňů 9, Prague

Short title
New Clearance Method for Hepatologic Diagnostics

Summary

PDD enables the conclusive of hemodynamic state as well as liver function. A repeated examination, even after a short pause (or under stress condition), enables to undemanding follow the dynamics of liver pathology. From presented parameters we have evaluated as reliable the *C5*-clearance, an expression of equilibrium state in the two compartment liver system. Further *T*-index expresses ratio *C5*/*CO*, it is a sensitive sign of the blood pole i.e. sinusoidal uptake in very good correlation with staging of hepatopaties. Within grading the isolated h-constant in correlation to *T*-index is valuable. The Japanese automatic analyser of ICG dilution and elimination curves, after incorporation of a two compartment mathematical model, becomes more useful for complex hepatological diagnostics.

Key words

Farmacokinetics, Steatofibrosis hepatis (SFH), Indocyanin green (ICG), two-compartment model, non-linear regression analysis, clearance value, relation to cardiac output (*T*-index).

Abstract

Non-invasive pulse dye densitometry is becoming of uppermost importance to clinical interest, to comparable results from other complicated and invasive examinations and may be, therefore, repeated in short time intervals for different indications with minimal stress of examined patient.

From presented parameters we have evaluated as reliable the *C5*-clearance, an expression of equilibrium state in the uptake and excretion liver system. Further *T*-index expresses ratio *C5* value to cardiac output, it is a sensitive sign of the blood pole i.e. sinusoidal uptake in very good correlation with staging of hepatopaties. Within grading the isolated h-constant in correlation to *T*-index is valuable. The Japanese automatic analyser of ICG dilution and elimination curves, after incorporation of a two compartment mathematical model, becomes more useful for complex hepatological diagnostics.

Introduction

Non-invasive pulse dye densitometry (PDD) is becoming of uppermost importance to clinical interest, to comparable results from other complicated and invasive examinations (Haruna *et al*. 1998) and may be, therefore, repeated in short time intervals for different indications (Imai *et al*. 1998) with minimal stress of examined patient (Mitchell *et al.* 1995), (Su *et al.* 1999), (Tichý and Loučka 2000).

The aim of this work is the analysis of dilution and elimination curves after single bolus injections of ICG (Iijima *et al.* 1998), (Yang *et al.* 1972). We have concentrated on the most sensitive clearance parameters for hepatological diagnostics (Shimizu *et al.* 1995), (Su *et al.* 1999) and on the relationship between simultaneously determined hemodynamic and clearance values. Further, using our mathematical model (Komenda and Tichý 1965), (Tichý and Komenda 1965), (Tsukada *et al.* 1996) we have compared ICG kinetic analysis, incorporated in Japanese automatic PDD analyser (NIHON-COHDEN).

Material and methods

PDD is carried out on bed-ridden patients on an empty stomach. Dilution and elimination curves describe the ICG concentration (mg/dm^3) dependence on time (t). The state of circulation is usually simultaneously evaluated bioimpedantionally by cardiodynamic data proceeding system (CDDPS) (Tichý 1995), with monitoring systemic blood tension such as mean pressure (*mBP*). Intravenous bolus is dosed from *25* to *50 mg* (average *38 mg*), applied in less than *20 s*. ICG concentration is monitored by nose or ear recorder (Mitchell *et al.* 1995), (Tichý and Loučka 2000) and determined values are compared, eventually corrigated with values estimated using direct photometry of at least two blood samples during elimination phase in a precisely determined time.

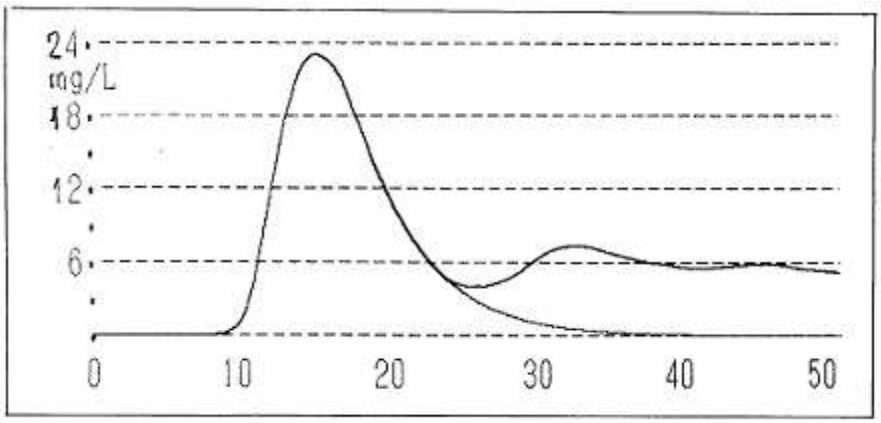

Fig.1 Dilution curve

Dilution curve is classically examined according to Hamilton (Tichý *et al.* 2005), (Yang *et al.* 1972) and enables cardiac output (*CO*) determination (Folkow and Neil 1971), (Yang *et al.* 1972) or, in relation to body surface area, cardiac index (*CI*). Correlation between hemodynamic parameters, determined by ICG dilution curves and by CDDPS is very satisfactory (Tsukada *et al.* 1996), however, in this work, it is not analyzed in detail. Appearance time (*AT*) is time interval between bolus application and appearance of curves ascendant branch, expression of circulatory velocity (Yang *et al.* 1972).

$$c(t) = \frac{g(t)}{\int_0^\infty g(t)dt} \qquad (1)$$

Dilution curve *c(t)* is proportional to circulation concentration of tracer, further treated by numerical integration according to Simpson's rule. The integral corresponds to area under this curve.

CO is determined from amount of given indicator (I_0), average concentration (*mP*) and mean transit time (*MTT*) (Folkow and Neil 1971)

$$CO = \frac{I_0}{mP \cdot MTT} \cdot 60 \qquad (2)$$

Dilution curves are monitored in one or two recirculatory peaks (Fig.1). Pathologic pattern of deformed curves in presence of intracardial shunts were not analysed. Concentration signal may be, with satisfactory precision approximation, considered as rectangular pulse (Jaroš 1998), (McBean and Rovers 1998).

Elimination curve (see Fig.2a) is given by gradual disappearance of ICG from blood, selectively through liver (Haruna *et al.* 1998), (Iijima *et al.* 1998), (Tichý and Loučka 2000). This process can be monitored from *3*. to *45*. minute. The initial phase, after ICG application, may be analysed by extrapolation or by calculation and virtual concentration *P(t)* in time *t = 0* may be defined. The ratio to the given dose of ICG (I_0) value *PV* (plasmatic volume) or *BV* (blood volume) is determined by recalculation (Iijima *et al.* 1998), (Schad 1987). In our examination the mean value of *PV*, in relation to body weight *55 ± 11 ml/kg*, is considered to be physiological and is comparable with other determination methods (Haruna *et al.* 1998).

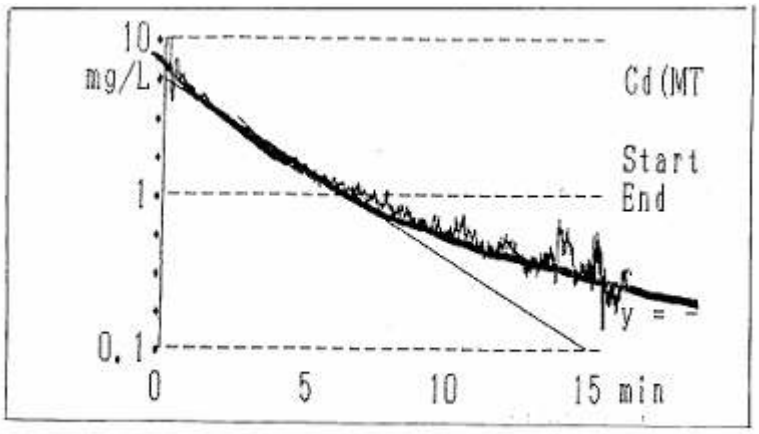

Fig.2a Elimination curve

Elimination curves analysis:

a) One parameter model:

The concept of a one-parameter model for ICG elimination is still being applied, including automatic analyser NIHON-COHDEN. This issues from the differential equation describing 1st order kinetics.

$$\frac{dI(t)}{dt} = -kI(t) \qquad (3)$$

For initial condition *t = 0, I(t) =* I_0 we obtain the following solution:

$$\frac{I(t)}{I_0} = e^{-kt} \tag{4}$$

assuming the complete distribution of given amount in plasmatic volume (*PV*) in concentration *P(0)*. The only diagnostic quantity is *k* constant here.

b) Two parameter model:

The process between blood and liver (on sinusoidal membrane) and than between liver cells and bile (on canalicular membrane) is taken into account (Komenda and Tichý 1965), (Tichý 1969). Agreement of digitalized curves (See Fig.2b) and its prediction, during utilization of a two parameter model, is very good (Tichý and Komenda 1965).

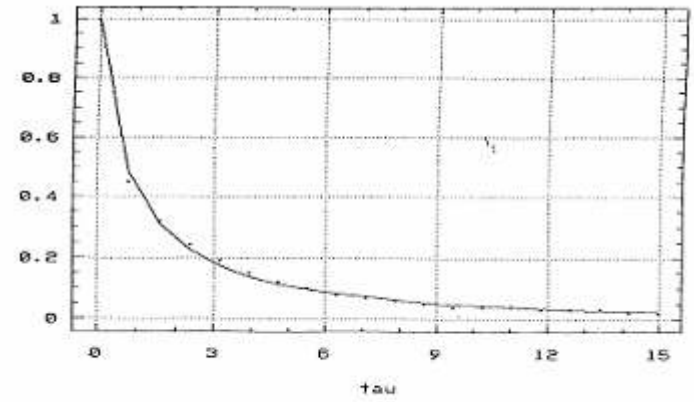

Fig.2b Digitalized elimination curve

Differential equation, describing the before proposed model (Tichý and Loučka 2000), (Tichý *et al.* 2005), has the following form:

$$\frac{dI(t)}{dt} = -kI(t)\left(1 - \frac{I_0}{H_0}\right) - \frac{k}{H_0} I^2(t) \tag{5}$$

Bernoulli equation of the 2nd order is under consideration where for initial condition $t = 0$, $I(t) = I_0$, it holds:

$$\frac{I(t)}{I_0} = \frac{\left(1 - \frac{I_0}{H_0}\right) e^{-k\left(1-\frac{I_0}{H_0}\right)t}}{1 - \frac{I_0}{H_0} e^{-k\left(1-\frac{I_0}{H_0}\right)t}} \tag{6}$$

When parameters are introduced it follows:

$$h = \frac{H_0}{I_0}, \quad \varphi = k\frac{h-1}{h} \tag{7}$$

then two parameter model is obtained in the following form:

$$p(t) = \frac{I(t)}{I_0} = \frac{h-1}{h(e^{kt}-1)} \tag{8}$$

where parameters are evaluated by non-linear regression. It is the way to obtain two independent characteristics *k* and *h* from data illustrated on the on the Fig.2b and to use whole part of curve for treatment.

Estimated values and indexes were statistically evaluated in relation to hepatological diagnosis, based on complex clinical and laboratory examination, including liver biopsy. This complex enables staging (*S*) and grading (*G*) of liver diseases (Lata *et al.* 2001), (Ludwig *et al.* 1995).

The clearance parameters according to following equations were calculated and their diagnostic sensibility was tested. For calculation of constants k and h the non-linear regression according to formula (8) Marquardt-Lenvenberg algorithm was used. From individual values *k*- and *h*-constants ensued diagnostic clearance parameters were according to the following equations (9) to (15):

$$C_1 = kI_m \frac{h \ln h}{h-1} \tag{9}$$

$$C_2 = \frac{2kI_m}{1-h+\sqrt{1+h^2}} \tag{10}$$

$$C_3 = 2kI_m \tag{11}$$

$$C_4 = \sqrt{2}kI_m \tag{12}$$

$$C_5 = \frac{kI_m h^2 \ln h}{h^2-1} = \frac{2C_1C_6}{C_3} \tag{13}$$

$$C_6 = kI_m \frac{h}{h+1} = C_3 \frac{h}{2(h+1)} \tag{14}$$

$$T = \frac{C5}{CO} \tag{15}$$

Ensemble *n=34* (males *31*,females *3*), average age *55,4* years (within age group *20* to *79*). Four patients were first examined while lying down and for the second time with equivalent ICG dose under continual stress of *60* to *70 w* in unchanged bed-ridden position for a period of *30* minutes.

(0) Physiological of liver, without evidence of liver disease, *n = 6*.

(1) Damage of liver (non-cirrhotic) is defined by different degrees of steatosis, fibrotization of periportal fields, eventually by portoportal septal formation.

Different celullisation in portobilliar fields and alteration of lobular structure (alcoholic $n = 12$ and non-alcoholic $n = 11$) were found. This concerns mainly patients with metabolic syndromes of diabetes type II, with insuline resistance, disorder of lipid metabolism, gout and morbid obesity (BMI ≥ *40*), totally, $n = 23$.

(2) Cirrhotic liver with evolution of portal hypertension is classified according to Child-Pugh's score (Ludwig 1995) in group A, B, and C, $n = 5$.

The activity of hepatocellular damage is evaluated and is taken into account with type and size of parenchymal cells necrosis in grading, including in the laboratory, findings especially aminopherazes level and hepatities markers. Method ANOVA (variance analysis (Jaroš 1998)), namely one-way method ANOVA and Kruskall-Wallis test (McBean and Rovers 1998), was used for statistical treatment. The same method was used for the statistical evaluation of the complex clinical state worth of all determined clearance parameters (see eqs. (9) to (15)). The proper *C5* value (equation (13)) has a great differential diagnostic ability, using from relations (Fig.3) and is statistically defined by *95%* confidence interval.

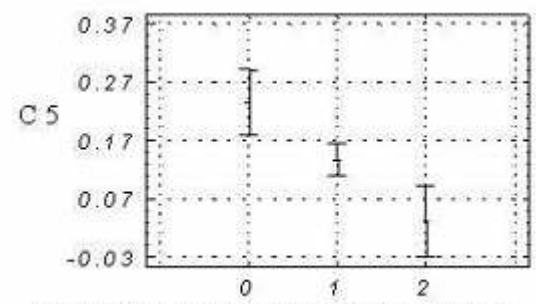

Fig.3 95% confidence intervals of *C5*

All given confidency intervals of mean values are disjunctive, differing three groups of hepatopathies presented above. The quantity k calculated from model represented by eq. (4) is not able to differentiate reliably this tree groups of hepatopaties (see Fig.4).

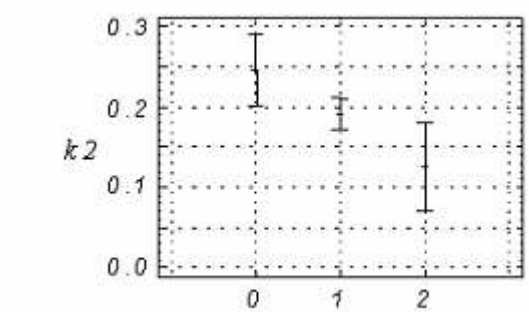

Fig.4 95% confidence intervals for k_2 value

Also *T*-index eq. (15) is considered to be clinically very important for even the proportionally heterogeneous non-cirrhotic reliably distinguished this group from cirrhotic, mainly A type (four-times) and C type (once).

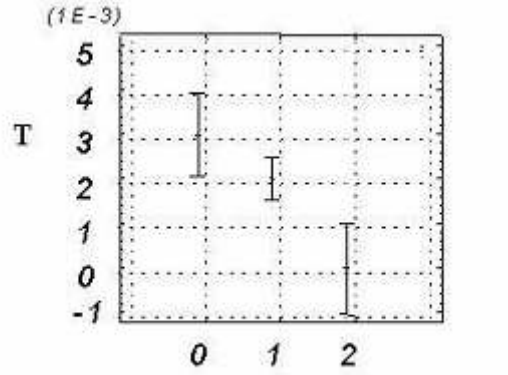

Fig.5 95% confidence intervals for *T*

The discrimination ability of *T*-index is also apparent during evaluation of the group (0), without conclusive hepatopathy (bioptically not verified), compared to alcoholic and non-alcoholic steatosis/steatofibrosis (1) (mostly bioptically verified).

Results and discussion

Priority is given to ICG clearance before the previously widely used bromsulphoftalein (BSP) (Komenda and Tichý 1965), (Tichý and Komenda 1965), for ICG does not cause anaphylactic or other allergic reactions. The availability of ICG is unfortunately limited in our country. The more complex the physiopathological knowledge of pharmacokinetics of used indicators, the richer the clinical experience, the more its diagnostic worth increases. ICG, but also BSP (when used for many years), enables to determine liver damage, its severity even though it has different ethiology (Lata *et al.* 2001). This was interpreted as functional blood pool failure of liver (on sinusoidal membrane), in compact relation to staging of liver diseases. For the activity evaluation i.e. grading, the correlation with presented clearance and *T*-index parameters is of low significance. For grading (functional state of canalicular membrane) is better expressed by isolated h-constant in relation to *C5* or *T*-values (see Fig.6).

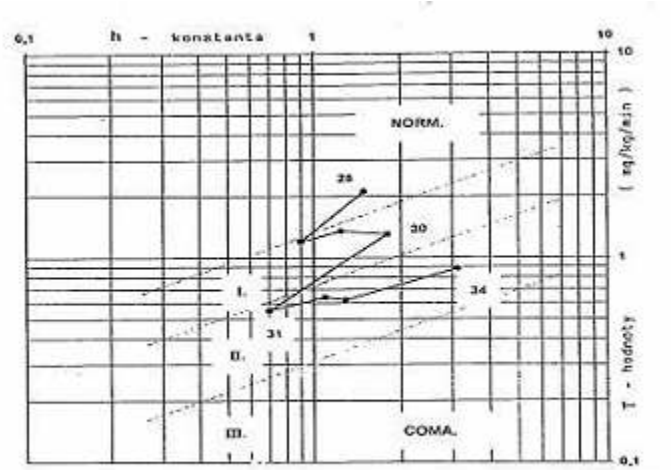

Fig.6 Evolution of ethylic hepatitis

Relation of *h*-constant and *T*-index during six years: very low *h*-constants in season of alcoholism followed by stepwise lowering of *T*-index.

The loss of ICG into other organs and spaces such as liver, especially into bone muscle and kidney, as evident from examination of tested animals, is negligible. Loss into urine in human beings is *0,2* to *1,9 %* (average *1,1 %*) (Mitchell *et al.* 1995), (Tichý and Loučka 2000), ICG is therefore, a vital dye appreciated in hepatology (Iijima *et al.* 1998), (Shimizu *et al.* 1995), (Tichý *et al.* 2005). The non-invasive method of PDD can be undemandingly repeated, eventually daily. It can be realized in bed-ridden patients or under stress and is able to follow the pathophysiological changes.

That, however, does not mean that such hypothesis can be rejected on the basis of our given data. However, the group of *34* persons (*42* examinations) seems to be, as far as volume information is concerned, based on extensive examination but from the rigorous statistics point of view is incomplete. We should especially point out the ratio between the range of individual groups and random selection of ill patients, that equivalent ratio between male and female patients is not observed and that patients do not cover the whole relevant age group. Remedy can, of course, be achieved only by increasing the ensemble, ensuring the attainability of ICG tracer.

Despite the diagnostic value of proposed parameters, especially *C5* clearance and *T*-indexes, exercising the liver clearance index *T* from minute volume which is simultaneously calculated from dilution and elimination curves is, on the basis of the already presented results, significant. We are able, on the basis of our data to determine disjunctive intervals well differ good liver function from ill ones at different stages of illness. When using PDD method we find it to be highly suitable

(not given problematic but also during development of liver damage of other etiology, eventually after liver transplantation and rejet syndrome), on the contrary to repeated needle biopsy.

Clinical diagnosis	**Assignment in Fig.**	***C5* confidence intervals 95%**
Healthy	**0**	**<0,17989 ; 0,29189>**
Steatosis and/or fibrosis	**1**	**<0,11192 ; 0,17041>**
Cirrhosis	**2**	**<0,00000 ; 0,09337>**

Table 1 *C5* boundaries determined for classification.

List of symbols

C	clearance values	[l/(60.s^{-1})]
H	relative liver capacity	[1]
$H(t)$	amount of tracer in the liver space	[mmol], [mg]
H_0	absolute liver capacity	[mmol], [mg]
$I(t)$	rest amount in blood	[mmol], [mg]
I_0	given dose of indicator	[mmol], [mg]
I_m	given dose related to body weight	[mmol/kg, mg/kg]
K	relative elimination rate	[(60.s^{-1})]
$p(t)$	relative plasma concentration	[1]
t	time	[(60.s)]
PV	plasmatic volume	[ml]
MV	minute volume	[ml/s]
CO	cardiac volume	[ml/s]
T	index	[1]
$C5$	clearance	[(60.s^{-1})]

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