Recommended Methods for Surface Counting to Determine Sites of Red Cell Destruction

A Report by the Panel on Diagnostic Applications of Radioisotopes in Haematology of the International Committee for Standardization in Hematology

=國文抄錄=

이 論文은 1973年 ICSH주최로 열린 panel에서 赤血球破壞 場所를 決定하기 위한 生體 體表計測法의 標準化에 關한 討論 結果를 抄錄한 것이다.

體表計測은 體外에서 計測器를 利用하여 各 臟器에서의 放射標識物質의 分布 및 時間經過에 따른 變化를 測定하는 것으로서 ^{51}Cr 를 使用하여 赤血球壽命을 測定할 때 肝, 脾, 心臟의 放射能을 計測한다. 이 方法은 各 臟器에서의 赤血球破壞의 程度를 예측할 수 있다. 特히 溶血性 貧血患者에서 脾臟摘出 여부를 決定하는데 도움이 된다.

이 panel에서는 主로 誤差의 原因이 되는 여러가지 要因에 對하여 討論하였으며 일반적으로 다음과 같은 것에 意見의 一致를 보았다. 即 脾臟의 位置는 ^{99m}Tc 로 脾走査를 실시하여 決定하는 것이 좋고, ^{51}Cr 은 體重 1 kg 당 1.5 μCi 를 사용하여, 計測器는 NaI crystal(직경이 5 cm 이상, 두께가 3.75 cm 이상)의 scintillation dector를 사용하고, 計測은 ^{51}Cr 로 標識된 赤血球 注入後 15分 以後에 하고 다음날 計測한 後 2週 동안에 적어도 6번 計測한다. Data 처리는 excess count 法과 脾와 肝의 比로서하는 것이 좋다.

1. INTRODUCTION

The expert panel on the Diagnostic Applications of Radioisotopes in Haematology, which was established by ICSH in 1966 has, at its meeting in Ulm(1972) and Leiden(1973), discussed recommendations for the standardization of in-vivo surface counting to determine the sites of red-cell destruction. The panel comprised E.H. Belcher(IAEA), N.I. Berlin(USA), J.G. Eernisse (Netherlands), L. Garby(Sweden), H. I. Glass(UK, Secretary), H. Heimpel(Federal Republic of Germany), M. Lee(Republic of Korea), S.M. Lewis(UK), P. Mollison(UK), E.A. Murphy(USA), Y. Najean(France) and L. Szur(UK, Chairman). In addition the Panel was assisted by A. Ganzoni(Switzerland) and K.E. Scheer(W.H.O). The following document was prepared at these meetings and deals with the technical and analytical aspects.

By "surface counting" is meant the use of external radiation detectors to determine the distribution of a radioactive tracer in the various organs of a subject and to study the change of distribution with time. Surface counting over the heart, liver and spleen, provides important supplementary data when red-cell survival studies are carried out with ^{51}Cr-labelled red cells.

The ultimate aim of this type of investigation is to determine the fraction of the red-cell destruction occuring in the organs especially in the spleen. Because of variation in the size of the organ and the rate at which ^{51}Cr is cleared from patient to patient, the counting rate at the surface may not have an exact relationship to the amount of radioactivity in the organ. Accordingly, only an approximate estimate of the extent of red-cell destruction can be obtained by this procedure. However, changes in counting

rate reflect changes in radioactivity within the organ and provide information on the relative role of each organ.

In clinical medicine the value of ^{51}Cr surface counting is to assess the main sites of destruction in haemolytic states, primarily in order to decide whether splenectomy would be of value. More information is available about the validity of prediction when splenic accumulation is high and splenectomy appears to be indicated than when it is not, as few clinicians have felt justified in carrying out the operation in the latter circumstances.

There is general agreement that despite a few false predictions the investigation is of value in forecasting the response to splenectomy especially in various cases of haemolytic anaemia, especially the acquired types. It must, however, be emphasised that in deciding whether splenectomy is indicated the clinical features and haematological and other laboratory data must also be taken into account.

Because even small technical variations may result in large discrepancies in surface counting measurements, it is of particular importance that a standardized method of ^{51}Cr surface counting should be adopted. The purpose of this document is to describe reference methods which take account of a number of factors which have been found to be causes of error. As the data obtained in surface counting studies depend on the detector system used and the points selected for counting, it will be necessary to define the significance of the data obtained with the standardized methods, before definite criteria for deciding on splenectomy can be established.

2. Methodology

2. 1. Localisation of spleen

Since the position of the spleen may vary widely between patients, the procedure requires the localisation of the organ in each individual subject. A widely used method is to determine the point at which the highest count rate is observed in the left hypochordium at 30~60 minutes after injection of the ^{51}Cr-labelled cells (see 2. 5 below). Subsequent measurements may be made at the same point or the point of maximal count-rate may be determined afresh on each occasion. There are, however, two possible objections to this method: (1) when the initial counting rate is relatively low there may be considerable error in determining the point at which the count-rate is greatest; (2) the initial radioactivity in the spleen reflects blood flow rather than the site at which red-cell destruction is maximal. For these reasons there may be an advantage in localising the spleen by scintigraphic visualisation following an injection of damaged red cells(e.g. heat-damaged) labelled with a minimal quantity of a short-lived radio-nuclide (e.g. ^{99m}Tc) and which preferably emits a gamma ray well below the 320 KeV gamma ray of ^{51}Cr.

2. 2. Injection of ^{51}Cr-labelled cells

Red cells should be labelled with ^{51}Cr using either method A or B as described in the ICSH Recommended methods for radioisotope red-cell survival studies.* It is advisable that the activity of the injected ^{51}Cr be near to the highest recommended, i.e. 1. 5μCi of ^{51}Cr per kilogram bodyweight, particularly when narrow collimation is used and when it is expected that the studies will take two weeks or longer. If a short-lived radio-nuclide is used for visualising the spleen the administration of ^{51}Cr-labelled red cells can only be carried out on the same day if the counting equipment can be adjusted to discriminate completely against the short-lived radio-

* Brit. J. Haemat(1971) **21**, 241, Amer. J. Clin. Path(1972), **58**, 71; Blood(1971) **38**, 378.

nuclide. Alternatively, the administration of ^{51}Cr-labelled red cells should be delayed for an appropriate period after the injection of the radionuclide.

2. 3. Detector System

A shielded collimated scintillation detector with a sodium iodide crystal of not less than 5 cm diameter and not less than 3. 75 cm thick should be used. If possible a dual detector system should be used. The collimator(s) should be of a single cylindrical hole type and details of the dimensions of a suitable collimator are given in the appendix(fig. 1). If a dual collimator system is to be used then the ends of the collimators should be 25~30 cms apart(Appendix, fig. 2). Each detector should be coupled to a pulse-height analyser and scaler. Investigations on children present special problems of positioning and radiation dose; smaller collimators must be used in this situation.

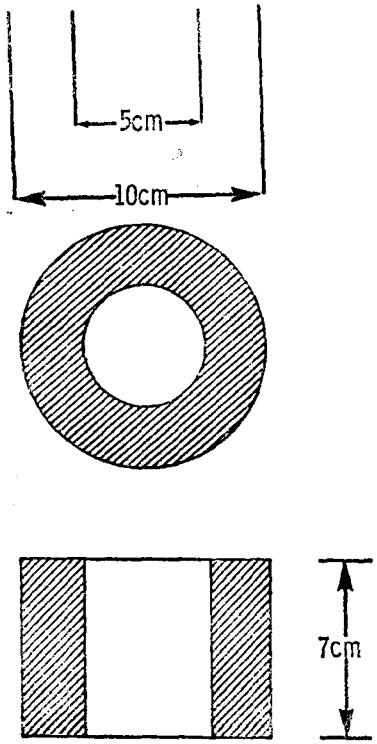

Fig. 1.

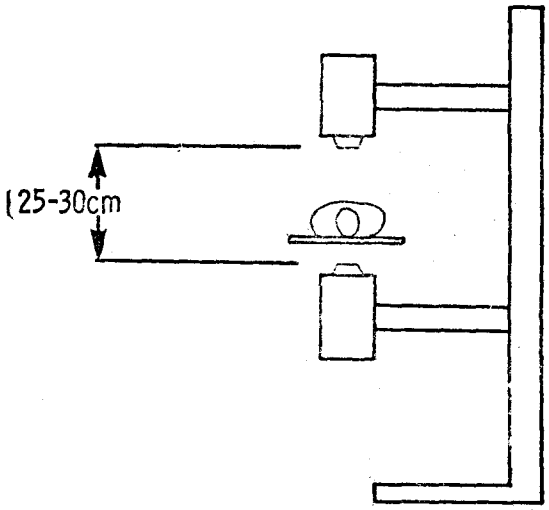

Fig. 2.

2. 4. Positioning of Detector(s)

Two counting procedures are acceptable, one using a dual detector system and another using a single detector.

(A). Dual Detector Method

The subject is placed in a supine position. The following points are marked on the skin with non-water-soluble marking ink.

Heart: third interspace at left sternal border.

Liver: halfway between midclavicular and anterior axillary lines on the right side of the body. In elderly subjects and in patients with chronic obstructive pulmonary disease the location of the liver should be checked by percussion.

Spleen: point of maximal activity (see 2. 1)

The counters are placed above and below the selected point in the same vertical axis and at a fixed distance from each other (Appendix, Fig 2).

(B) Single Detector Method

For measurement of heart and liver the subject is placed in a supine position, and the following points are marked on the skin with non-water-soluble marking ink.

Heart: in the midline at the level of the third interspace.

Liver: as for the dual detector method.

The detector is placed vertically with the centre of the collimator over the selected point; and with the collimator just touching the skin.

Spleen: For measurements over the spleen the detector is placed in a horizontal position; the point of maximal activity is determined(see 2. 1) and the collimator is placed over that point, just touching the skin.

To avoid fluctuations in the positions of the organs, measurements should, if possible, be carried out no each occasion at the same time of day, and great care must be taken to ensure that the collimator(s) are placed precisely over the chosen points and that the subject is maintained in the same posture on each occasion. To ensure that the surface markings remain in situ they should be covered by non-allergic tape or transparent plastic dressing.

2. 5. Measurements

The first measurements (Day 0) should be carried out at least 15 minutes after the injection of ^{51}Cr-labelld cells, in order to allow for adequate mixing. In patients with splenomegaly, measurement should be delayed for 60 minutes. Information on distribution of blood in the spleen may be obtained by recording counts during the mixing period, but details of such studies fall outside the scope of the present document. Subsequent measurements are carried out on Day 1 and thereafter not less than six times during the following two weeks. Daily counts are indicated in sevese haemolytic states.

Before surface counting is started on each occasion the patient should be in a resting state. Not less than 2,500 counts should be recorded at each site. Preferably duplicate measurement (i.e. 2,500 counts each) should be carried out and summated; if the counts differ by more than the SE (i.e. $2.8\sqrt{n}$) they must be repeated. A standard of ^{51}Cr (e.g. 1μCi) should be measured, under conditions of constant geometry, in order to check the instrument performance and to correct for radioactive decay.

3. 1. Calculation and Presentation of Data

The counts obtained over each organ should first be corrected for background, physical decay and instrument performance using the standard (see 2. 5). When a dual detector system is used the corrected counts are summated. There are several methods for presentation of data which are in general acceptable. The "excess counts" method is particularly useful as it can be used in investigations which aim at the absolute quantitation of the fraction of red cells destroyed in the spleen when scanning and computer facilities are available. Accordingly, this method is proposed as the reference method. If another method is used the results should additionally be expressed as "excess counts" in order to facilitate comparison or results.

Excess Counts Method

The counting rate over the heart might be expected to fall proportionately at the same rate as that in the blood, and if there were no deposition of ^{51}Cr in the spleen or liver the counting rate over these organs would also be expected to fall in a similar manner as that in the blood. In practice the fall over the heart is slightly slower, presumably owing to some accumulation of ^{51}Cr in cardiac or neighbouring tissues. In very rare cases in which radioactivity

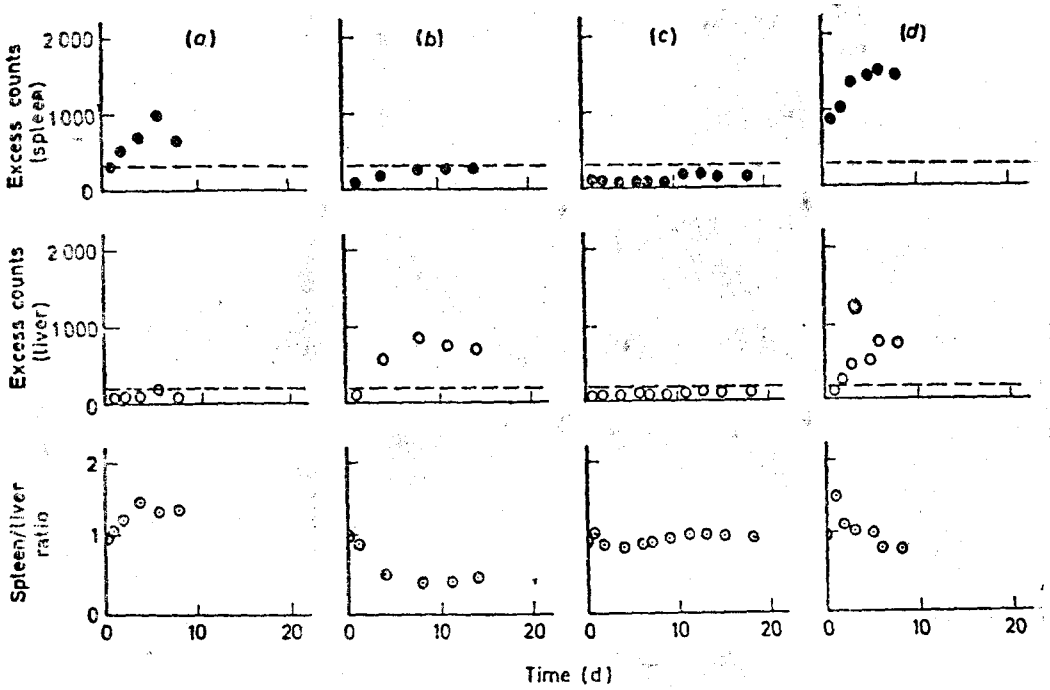

Fig. 3. Patterns of accumulation of ^{51}Cr in the spleen and liver of patients with haemolytic anaemia: (a) excess accumulation in spleen alone (hereditary spherocytosis); (b) excess accumulation in liver alone (sickle-cell diseaes): (c) no excess accumulation in either spleen or liver (hereditary non-spherocytic anaemia); and (d) excess accumulation in both liver and spleen (auto-immune haemolytic anaemia).

accumulates in the lungs (e.g. in haemosiderosis) the "heart" counts will fall even more slowly than the counts in the blood, and it may not be possible in such cases to apply the standard procedure. Any accumulation of ^{51}Cr over liver or spleen in excess of that over the heart is taken to indicate destruction of red cells in that organ.

The initial counts over the heart are designated as 1,000. The factor required to convert the observed counting rate over the heart to 1,000 is applied to all other observed counts of spleen and liver as well as the heart; the method is illustrated in Table I. These counts are then plotted against time.

Spleen: Liver ratio

An additional parameter of value in the interpretation of results is the spleen: liver ratio. This is calculated simply as the ratio of the observed count rate over the spleen to that over the liver both corrected for background and radioactive decay. The ratio is also plotted against time; additionally, to facilitate comparison of results in different patients the ratio on Day 0 may be taken as 1.0 and subsequent ratios expressed proportionately. The pattern of results of surface counting studies obtained in various conditions are illustrated in Appendix, fig 3.

As far as possible the actual counting rates over heart, liver and spleen, corrected for background and radioactive decay, should be given in published reports. If this is not feasible, at least the following parameters should be included: (a) Excess counts (spleen and liver) at T

50 ^{51}Cr(if measured), and at their maxima as obtained from a smoothed curve through all points; (b) spleen: liver ratio on Day 0, at T50 ^{51}Cr, and its maximum as obtained from a smoothed curve through all points.

It should be emphasised that the data must be interpreted overall and no reliable deduction can be made from a single measurement.

3. Radiation Dosimetry

See Table III of ICSH Recommended Methods for Radioisotope Red Cell Survival Studies.

The actual count rate over the heart on Day 0 was 7,500 counts per minute. This was recorded as 1,000 and all other counts were adjusted proportionately.

Table 1. Example of Method for Calculation of Surface Counting Data

Day	0	1	2	5	8	10	12	14
Heart	1,000	850	780	720	670	600	500	370
Liver								
Actual counts	670	670	660	560	640	630	550	530
Expected counts		570	522	482	449	402	335	248
Excess		100	138	78	191	228	215	282
Spleen								
Actual count	970	1,265	1,490	1,800	2,130	2,370	2,210	2,020
Expected counts		825	755	700	650	580	485	360
Excess		440	734	1,102	1,480	1,788	1,725	1,661
Spleen: liver ratio								
Actual*	1.45	1.89	2.26	3.21	3.33	3.76	4.02	3.81
Adjusted	1.00	1.30	1.56	2.21	2.31	2.59	2.76	2.62

* Obtained from the actual counts of the organs. The ratio obtained on Day 0 was recorded as 1.00 and the results on subsequent days were adjusted proportionately.