Chapter 5

Discussion & conclusions

The chapter deals with discussion and conclusions. The thesis ends with this chapter. The chapter interprets the results of the investigations on the clinical and biophysical parameters of the blood on cancer patients in comparison with healthy persons. It gives information on importance of various clinical and biophysical parameters in the proper identification of the disease. The results of the investigation have been discussed correlating with biological and medical aspects. At the end of the chapter, important conclusions are drawn from the investigations presented.
All living things are composed of cells which are the basic building blocks of life. Every part of the body from the brain to the blood and from bones to the glands is made up of colonies of cells. In an adult organism each and every cell has a set of chromosomes that contain, in code form, the complete genetic information to specify the size and shape and nature of that organism.

The number of chromosomes per cell varies with the species. In the human, a normal cell contains 46 chromosomes. The first cell of a new human being contains 23 chromosomes donated by mother and an equal number by the father. This single cell produced at conception, replicates or divides to form two cells which divide further forming four cells and so on.

At the later stage some cells differentiate and become specialized, capable of performing a specific function but unable to divide again. In the course of time a mature adult organism is formed composed of populations of dividing and differentiated cells.

These biological cells are highly complex in nature and contain a large number of organelles to carry on different life processes. Structurally human beings have a fluid connective tissue with cells such as erythrocytes (RBC), leukocytes (WBC), thrombocytes or platelets and the liquid matrix called plasma.
As is known, cancer is a cellular genetic disease, mainly caused by uncontrolled fast growth of abnormal cells of any kind. In the study of the disease of peculiar nature – the cancer, whose root cause is not known till today, there are two aspects to be considered seriously. One is proper diagnosis of the disease and second is how best one can control the multiplication of cancer cells.

As far as diagnosis is concerned histopathological and biochemical approaches are widely being adapted, while biophysical methods are neglected to some extent.

Now, coming to the aspect of treatment of cancer, there are basically two approaches – Physical (Radiation therapy) and chemical (Chemotherapy).

In the cancer study, one has to take into account the very important connective tissue - the blood. Then there arise two questions.

1. Whether the abnormal changes (Cancer) occurred in any tissue are reflected or mirrored in the blood to be helpful for the diagnosis?

2. Whether the radiation therapy has any adverse effects on the blood? If yes, the blood rheology will be affected, leading to cardiovascular complications.
Hence, outcome of the thesis is an attempt to answer the above questions, which escaped from the medical discipline. In view of this, in the present investigation, the physical parameters – viscosity, surface tension, volume flow rate, erythrocyte size, refractive index, electrical conductivity, excess permittivity and pH have been selected to assess the degree of influence of cancer as well as radiation therapy on blood and its constituents.

The present study has the genesis to achieve this objective. This study has been in the following types of cancer. *Carcinoma cervix, breast, rectum, head neck, lung, bladder, vagina, penis, brain etc.*

The blood samples are collected from the normal persons and cancer patients suffering from the above mentioned types and various stages and it is subjected to above clinical and biophysical parameters.

### 5.1. Clinical parameters – Blood Tests

Blood is used as diagnostic parameter for identifying many diseases. The complete blood picture (CBP) is the most common test which gives the details of total number of RBC, WBC and platelets.

CBP is usually prescribed by a physician as a clinical parameter to diagnose any disorder in the number of RBC, WBC and platelets. RBC Count, hemoglobin content, mean cell volume gives us the details
regarding the oxygen carrying capacity of the blood and also suggests the general condition of the patient.

Since WBC helps in fighting the disease causing germs in the body, any increase in the number of WBC indicates general body infection, such as urine infection caused by bacteria etc. In the same way if the platelet number is too low or lower than normal person, the patient must be bleeding in any part of the body as platelets help in clotting of blood.

The mean values of Hemoglobin (Hb) content for normal blood is 13.9 g/dl and for the cancer blood is 11.2 g/dl before commencement of radiation treatment and 10.9 g/dl after completion of radiation treatment, (Fig. 5.1.).

From the above data, one can say that there is a decrease in the mean values of Hb content in the cancer patient before and after radiation treatment when compared with the mean values of normal persons.

Hence, one can infer that the Hb content has decreased in the cancer patients due to the following observations.

a) A bone marrow region around the tissues for which radiation is given is affected. Hence bone marrow’s RBC production capacity is also affected.
b) The second observation can be: as the cancer cells are less adhesive in nature, they enter into the blood stream easily and they demand more protein from the body. Hence the blood protein and the Hb content sufficiently decreased in their counts.

The RBC count for normal blood is 4.91 millions/cumm and for the cancer blood is 4.03 millions/cumm before commencement of radiation treatment and 3.93 millions/cumm after completion of radiation treatment, (Fig. 5.1.). From the data, one can say that there is a decrease in the mean values of RBC count in the cancer patient before and after radiation treatment when compared with that of normal persons, which is due to the reduction in hemoglobin content.

The WBC count for normal blood is 6345 cells/cumm and for the cancer blood is 8946 cells/cumm before commencement of radiation treatment and 5908 cells/cumm after completion of radiation treatment.

From the data, it is obvious that there is an increase in the mean values of WBC count in the cancer patient before radiation treatment and decreased after radiation treatment when compared with the mean values of normal persons, (Fig. 5.1.). There can be an infection which is verified from the case sheets of the patients, because increase in WBC count signifies infection in the body. But after radiation treatment, the WBC count came down to the normal value as the patient started responding to the treatment.
The platelet count for normal blood is 2.61 lakh/cumm and for the cancer blood is 2.96 lakh/cumm before commencement of radiation treatment and 2.46 lakh/cumm after completion of radiation treatment.

From the data it is seen that the platelet count increases in cancer patient but after radiation treatment it comes down to normal level. This shows platelet count also a clinical parameter by which disease can be identified, (Fig. 5.1.).

The ESR of normal blood is 12 mm while in the case of cancer it is 64.31 mm before commencement of radiation treatment and 56.5 mm after completion of radiation treatment.

From the data it is seen that there is a great variation in the ESR values of normal and cancer blood which is usually expected off. Any disease in the body leads to increase in the ESR values which decrease as a response to radiation treatment. Hence ESR can also be considered basic parameter for identifying the disease, (Fig. 5.1.).
Fig. 5.1. Clinical parameters

**HEMOGLOBIN (g/dl)**

<table>
<thead>
<tr>
<th>Content</th>
<th>16</th>
<th>14</th>
<th>12</th>
<th>10</th>
<th>8</th>
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</thead>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>11.2</td>
<td>10.9</td>
<td></td>
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</tr>
</tbody>
</table>

**RBC Counts (mill/cumm)**

- **NORMAL**
- **BEFORE RT**
- **AFTER RT**

<table>
<thead>
<tr>
<th></th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
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<tbody>
<tr>
<td>4.91</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4.03</td>
<td></td>
<td></td>
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<tr>
<td>3.93</td>
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</tr>
</tbody>
</table>
Fig. 5.1. Clinical parameters (contd)

![Bar chart showing WBC Counts (cells/cumm) and PLATELET Counts (lakh/cumm)]
Fig. 5.1. Clinical parameters (contd)
5.2. Biophysical parameters

Apart from measuring the physical composition of the blood, the other properties such as pH, viscosity, volume flow rate, surface tension, refractive index, electrical conductivity, dielectrophoretic collection rate, excess permittivity are also examined to identify or diagnose a disease in particular, cancer which is the present topic of the thesis “Biophysical investigations on blood of cancer patients”.

The size of erythrocytes of normal blood is 7.96 μm and of cancer is 9.09 μm before commencement of radiation treatment and 8.65 μm after completion of radiation treatment. From the data, one can observe that there is an increase in the size of RBC in the cancer patient but after radiation treatment a decrease in the size is seen.

The increase in the size of RBC may be due to hypotonic nature of the blood in the cancer patient due to which the RBC swells up. But once the treatment starts the size moves to normal showing the positive response towards treatment. Hence size of erythrocytes can be taken as a reliable parameter to identify the disease in a person, (Fig. 5.2.).

The refractive index of blood plasma of normal person is 1.347 and for the cancer blood is 1.347 before commencement of radiation treatment and 1.346 after completion of radiation treatment.
From the data it can be seen that there is no noticeable change in the refractive index of the blood for normal and cancer patients, (Fig. 5.3.).
Fig. 5.2. Size of erythrocytes (RBC) in μm

Fig. 5.3 Refractive index
Viscosity means thickness or resistance to flow. Blood is about 3 to 5 times thicker than water. This is due to the proteins present in the plasma and this thickness also contributes to the blood pressure.

The coefficient of viscosity for normal blood is 0.065 poise and of cancer blood, it is 0.067 poise before commencement of radiation treatment and 0.071 poise after completion of radiation treatment.

No significant variation is seen in the cancer blood compared to normal. This is a positive sign as the blood may get coagulated with higher viscosities which may lead to cardiovascular problems in the patients. Similarly, viscosity of plasma also shows a mean value of 0.024 poise for a normal person, while it is 0.027 poise for a cancer patient before radiation treatment and 0.026 poise after radiation treatment.

These values also suggest there is a considerable increase in the mean values of cancer blood which come down after radiation treatment. Hence, one can infer that viscosity may also be taken as a biophysical parameter to identify the cancer disease in a cancer patient, (Fig. 5.4.).

The percentage of variation in viscosity in blood and plasma of cancer patients before and after radiation treatment shows significant variation in some cases which may be attributed to depletion of nutrients and proteins together with reduction in the RBC count which in turn influences other parameters like velocity and volume flow rate.
Volume flow rate is defined as the volume flow divided by the cross sectional area. The characteristic velocity is the maximum velocity of blood flow in a capillary tube. It is a characteristic parameter. Hence volume flow rate is the volume of the blood that flows per second in a capillary tube that indicates any abnormality in the blood.

Volume flow rate for normal blood is 5.35 cc/sec and for the cancer blood is 5.16 cc/sec before commencement of radiation treatment and 4.97 cc/sec after completion of radiation treatment.

Volume flow rate for normal plasma is 13.9 cc/sec and for the cancer plasma is 12.48 cc/sec before commencement of radiation treatment and 12.98 cc/sec after completion of radiation treatment.

The volume flow rate of cancer blood decreases both in blood and plasma compared to normal person. But in the case of blood, the volume flow rate falls similar to ESR which is a positive sign showing response to the treatment, (Fig. 5.4.).

Since any mechanical system tries to come back to a state of minimum potential energy, the free surface of the liquid also tries to do the same. In this process a contraction in the area takes place. As a result the free surface experiences a tension which is nothing but surface tension of the liquid. In case of cancer patients any abnormality noticed in the blood will help in the proper diagnosis.
The surface tension for normal blood is 52.67 dyne/cm and for the cancer blood is 49.57 dyne/cm before commencement of radiation treatment and 53.41 dyne/cm after completion of radiation treatment.

From the data, one can infer that the mean values of surface tension of blood (49.59 dyn/cm) and plasma (50.77 dyn/cm) decrease by almost 6% which is a very rare observation in a cancer patient but after RT the surface tension values of blood (53.41 dyn/cm) and plasma (53.43 dyn/cm) increased matching the values of the normal person’s blood and plasma.

Though there is a great variation in the mean values of normal and cancer blood, after radiation treatment there is an increase in the mean values of surface tension of the cancer blood. Hence this can be used as a reliable criterion to differentiate the normal blood from the cancer blood, (Fig. 5.4.).
Fig. 5.4. Viscometric parameters

**Viscosity of Blood (poise)**

**Viscosity of Plasma (poise)**

- Normal
- Before RT
- After RT
Fig. 5.4. Viscometric parameters (contd)

VOUME FLOW RATE OF BLOOD (cc/sec)

VOLUME FLOW RATE OF PLASMA (cc/sec)

NORMAL

BEFORE RT

AFTER RT
Fig. 5.4. Viscometric parameters (contd)

- **Surface Tension of Blood** (dyn/cm)
  - Normal: 52.67 dyn/cm
  - Before RT: 49.57 dyn/cm
  - After RT: 53.41 dyn/cm

- **Surface Tension of Plasma** (dyn/cm)
  - Normal: 55.23 dyn/cm
  - Before RT: 50.77 dyn/cm
  - After RT: 53.43 dyn/cm
The electrical conductivity for normal blood is 3.93 mS and for the cancer blood is 3.434 mS before commencement of radiation treatment and 3.45 mS after completion of radiation treatment. The electrical conductivity for normal plasma is 7.712 mS and for the cancer plasma is 7.609 mS before commencement of radiation treatment and 7.651 mS after completion of radiation treatment.

No significant change is seen in the EC of blood for the cancer patient before and after RT. Blood maintains normal concentration even after radiation treatment which can be taken as a positive sign as the blood and plasma do not coagulate after receiving radiation. This may avoid many cardiovascular problems in the patient, (Fig. 5.5.).
Fig. 5.5. Electrical conductivity

**ELECTRICAL CONDUCTIVITY OF BLOOD**

- 3.931
- 3.434
- 3.45

**ELECTRICAL CONDUCTIVITY OF PLASMA**

- 7.712
- 7.609
- 7.651

Legend:
- **NORMAL**
- **BEFORE RT**
- **AFTER RT**
pH signifies the hydrogen ion concentration. The normal pH range of the blood is 7.35 to 7.45 which makes the blood slightly alkaline.

The pH for blood and plasma for normal person are 7.36 and 7.375, for the cancer blood 7.34 and 7.192 before commencement of radiation treatment and 7.127 and 7.137 after completion of radiation treatment respectively, (Fig. 5.6.)

Dielectrophoresis of living cells is concerned with the rate at which the biological cells in a given suspension collect when non-uniform electric field is applied. The rate at which the cells gather at electrodes in alternating applied electric field is called dielectrophoretic collection rate (DCR). The present investigation is concerned with non-uniform electric fields and their interactions with human erythrocytes in order to understand how electrical properties of erythrocytes from patients suffering from different cancers are influenced by cancer at different levels.

For this purpose, the dielectrophoretic technique is adopted. It is a technique to serve as a potential tool to sense the subtle changes in electrophysiology of erythrocytes. To some extent it helps in monitoring the disease through dielectrophoretic behaviour of erythrocytes as these properties help the blood to carry out the life processes.
The dielectrophoretic collection rate (DCR) for normal blood is 83.5 μm/min and for the cancer blood is 119 μm/min. The excess permittivity, $K_e$ for normal blood is 2.32 and for the cancer blood is 4.81.

There is a significant variation in DCR and $K_e$ of blood of cancer patient when compared to normal person, which can be attributed to different composition and electrical makeup, the suspension medium being the same. This can be taken as a positive symptom to identify and differentiate the blood of normal and cancer patient. Also, can be a reliable biophysical parameter for diagnostic purposes, (Fig. 5.7.).
Fig. 5.6. pH

**pH of Blood**

**pH of Plasma**
Fig. 5.7. Dielectrophoretic parameters

**Dielectrophoretic Collection Rate (μm/min)**
- Normal: 83.5
- Before RT: 119.2

**Excess Permittivity (Ke)**
- Normal: 2.32
- Before RT: 4.81
Apart from clinical parameters, biophysical parameters help us to assess cancer invasion on different tissues in the human body, mirrored in physical properties of blood. Since the discovery of disease cancer, it is diagnosed solely by histopathological and biochemical approach.

This research also enabled us to examine whether there are any adverse effects on the ionizing radiations on blood so as to take necessary remedial steps. The biophysical parameters such as viscosity and electrical conductivity rule out the effects of ionizing radiation on the blood. Because the results of viscosity and electrical conductivity prove that the composition of blood has not changed even after high dose of radiation which may coagulate the blood and cause cardiovascular problems to the patient.

Finally, according to the observations drawn from the data collected from normal and cancer patients, it is concluded and verified that biophysical examinations can be complimentary to biochemical approach in the diagnostic process.

From the present investigation, following conclusions can be drawn

1. In most of the cancer patients, RBC count, and Hemoglobin content considerably decrease, while WBC count and platelet count increases.
2. ESR increases significantly, and hence can serve as a potential tool for the diagnosis of cancer.

3. In cancer, there is an increase in size of RBC of cancer blood.

4. The rheological parameters are not affected much adversely due to cancer, otherwise, severe cardiovascular complications may be expected.

5. In cancer, the electrical make up of the blood and its plasma is not perturbed much, avoiding coagulation of cells as well as cardiovascular implications.

6. Electrical make up of RBC is altered significantly due to cancer, as revealed by DCR and $K_e$. Hence, dielectrophoresis is a sensitive biophysical technique to detect cancer at cellular level.

7. Clinical parameters are influenced by radiation treatment.

8. Radiation therapy does not influence much the rheological parameters. This is the important finding of the present study.
Table 5.1. A comparison on Clinical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Cancer Before RT</th>
<th>Cancer After RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl) content</td>
<td>13.9 ± 2.02</td>
<td>11.2 ± 2.0</td>
<td>10.9 ± 1.9</td>
</tr>
<tr>
<td>RBC count (millions/cumm)</td>
<td>4.91 ± 0.24</td>
<td>4.03 ± 0.57</td>
<td>3.93 ± 0.6</td>
</tr>
<tr>
<td>WBC count (cells/cumm)</td>
<td>6345 ± 1149</td>
<td>8946 ± 3475</td>
<td>5908 ± 2603</td>
</tr>
<tr>
<td>Platelet count (lakh/cumm)</td>
<td>2.61 ± 0.80</td>
<td>2.96 ± 1.10</td>
<td>2.46 ± 0.9</td>
</tr>
<tr>
<td>ESR value (mm)</td>
<td>21 ± 12</td>
<td>64.31 ± 24.27</td>
<td>56.5 ± 28.65</td>
</tr>
</tbody>
</table>

Table 5.2 A comparison on Size of erythrocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Cancer Before RT</th>
<th>Cancer After RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (μm)</td>
<td>7.96 ± 0.39</td>
<td>9.09 ± 0.81</td>
<td>8.65 ± 0.59</td>
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</table>

Table 5.3 A comparison on Refractive index

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Cancer Before RT</th>
<th>Cancer After RT</th>
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<tr>
<td>Refractive index</td>
<td>1.347 ± 0.002</td>
<td>1.347 ± 0.003</td>
<td>1.346 ± 0.001</td>
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Table 5.4. A comparison on viscometric parameters

<table>
<thead>
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<th>Parameter</th>
<th>Normal</th>
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<th>Cancer After RT</th>
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</thead>
<tbody>
<tr>
<td><strong>Surface tension (dyn/cm)</strong></td>
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<tr>
<td>Blood</td>
<td>52.67</td>
<td>49.59</td>
<td>53.41</td>
</tr>
<tr>
<td>± 4.62</td>
<td></td>
<td>± 4.85</td>
<td>± 6.23</td>
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<tr>
<td>Plasma</td>
<td>55.33</td>
<td>50.77</td>
<td>53.43</td>
</tr>
<tr>
<td>± 5.04</td>
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<td>± 4.16</td>
<td>± 5.07</td>
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<td><strong>Viscosity (poise)</strong></td>
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</tr>
<tr>
<td>Blood</td>
<td>0.065</td>
<td>0.067</td>
<td>0.071</td>
</tr>
<tr>
<td>± 0.014</td>
<td></td>
<td>± 0.012</td>
<td>± 0.017</td>
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<tr>
<td>Plasma</td>
<td>0.024</td>
<td>0.027</td>
<td>0.026</td>
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<tr>
<td>± 0.002</td>
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<td>± 0.003</td>
<td>± 0.002</td>
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<tr>
<td><strong>Volume flow rate (cc/sec)</strong></td>
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<tr>
<td>Blood</td>
<td>5.35</td>
<td>5.16</td>
<td>4.97</td>
</tr>
<tr>
<td>± 1.12</td>
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<td>± 0.82</td>
<td>± 1.27</td>
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<tr>
<td>Plasma</td>
<td>13.90</td>
<td>12.48</td>
<td>12.98</td>
</tr>
<tr>
<td>± 1.40</td>
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Table 5.5 A comparison on Electrical conductivity ($\sigma$)

<table>
<thead>
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<th>Parameter</th>
<th>Normal</th>
<th>Cancer Before RT</th>
<th>Cancer After RT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrical conductivity (mS)</strong></td>
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</tr>
<tr>
<td>Blood</td>
<td>3.931</td>
<td>3.434</td>
<td>3.450</td>
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<tr>
<td>± 0.523</td>
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<td>± 0.466</td>
<td>± 0.534</td>
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<tr>
<td>Plasma</td>
<td>7.712</td>
<td>7.609</td>
<td>7.651</td>
</tr>
<tr>
<td>± 0.584</td>
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<td>± 0.254</td>
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Table 5.6. A comparison on pH

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</tr>
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<td><strong>pH</strong></td>
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<tr>
<td>Blood</td>
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<td>7.240</td>
<td>7.127</td>
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<tr>
<td>± 0.208</td>
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<td>± 0.162</td>
<td>± 0.153</td>
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<tr>
<td>Plasma</td>
<td>7.375</td>
<td>7.192</td>
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<tr>
<td>± 0.222</td>
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<td>± 0.238</td>
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Table 5.7. A comparison on dielectrophoretic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Cancer</th>
</tr>
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<tbody>
<tr>
<td>Dielectrophoretic collection rate (μm/min)</td>
<td>erythrocytes 83.5 ± 8.18</td>
<td>119.2 ± 20.84</td>
</tr>
<tr>
<td>Excess permittivity (K&lt;sub&gt;e&lt;/sub&gt;)</td>
<td>erythrocytes 2.32 ± 0.44</td>
<td>4.81 ± 1.75</td>
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