CHAPTER 2

DIAGNOSTIC METHODS FOR RHEUMATOID ARTHRITIS

The summary of the various methods available in the diagnosis of RA is given in Table 2.1. These methods are broadly classified as the following: i) biochemical method ii) x-ray method iii) biopsy/autopsy method and iv) research method. According to Indian Rheumatology Association (IRA) consensus report (2008), both the biochemical method and x-ray method have been considered as ‘gold’ standard method in the evaluation of RA [42]. Although both the biochemical method and x-ray method are gold standard for diagnosis of RA, these method has the following shortcomings: i) The biochemical method may not give complete clinical information about the progression of the disease; ii) X-ray method is not able to detect early disease manifestations such as inflammatory changes in the soft tissues (synovitis, tensynovitis, enthesitis etc.) and bone erosion. Hence new methods such as MRI, thermal imaging and ultrasound are required in the evaluation of RA.

2.1 BIOCHEMICAL METHOD

The commonly used biochemical test for diagnosis of RA includes ESR, CRP, RF, complete blood count such as hemoglobin and white blood cells (WBC). The ESR is widely used as an indicator for inflammation. It is measured by using automated ESR analyser (Bestmatic cube 30, Transasia, Germany). It measures how fast red blood cells fall to the bottom of a fine glass tube that is filled with the patient’s blood. The ESR is often increased to greater than 20mm per hour, which may be used to monitor the disease development. High level of CRP(>80 mg/dl) is also indicator of active inflammation [43]. The antibodies that collect in the synovium of the joint are known as rheumatoid factor (RF). It is a type of protein synthesized by the immune system. An increased level of the RF factor (>20 IU/ml) is the indicative of RA. Levels of CRP and RF is measured by immunoturbidimetric
### Table 2.1 Summary of various methods for diagnosing RA

<table>
<thead>
<tr>
<th>I. Biochemical Method</th>
<th>II. X-ray Method</th>
<th>IV. Research Method</th>
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<tbody>
<tr>
<td>- Gold Standard Method</td>
<td>- Gold Standard Method</td>
<td>- HAQ –DI score (0-4) [50]</td>
</tr>
<tr>
<td>i. ESR</td>
<td>Anterior-posterior (AP) view and Posterior-anterior (PA) view.</td>
<td>- DAS28 score (0-10) [50]</td>
</tr>
<tr>
<td>- RA&gt;20mm/hour</td>
<td></td>
<td>b. Central / Peripheral Dual energy X-ray Absorptiometry (DXA) [25]</td>
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<tr>
<td>ii. CRP</td>
<td></td>
<td>- Whole body BMD (g/cm²)</td>
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<tr>
<td>- RA &gt;80mg/dl</td>
<td></td>
<td>- Regional BMD (g/cm²)</td>
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<tr>
<td>iii. RF</td>
<td></td>
<td>- Forearm , Spine, Hip</td>
</tr>
<tr>
<td>- RA&gt;20U/ml</td>
<td></td>
<td>c. Thermography [37]</td>
</tr>
<tr>
<td>- Total WBC count</td>
<td></td>
<td>- Temperature indices:</td>
</tr>
<tr>
<td>- RA&gt;10,000 counts/ml</td>
<td></td>
<td>- Skin temperature (°C)</td>
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<tr>
<td>b. Advanced biomarker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Anti-cyclic citrullinated peptide antibody</td>
<td></td>
<td>- Heat distribution index</td>
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<tr>
<td>- RA&gt;25U/ml [43]</td>
<td></td>
<td>- Thermographic index</td>
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<tr>
<td>III. Biopsy / Autopsy</td>
<td></td>
<td>d. Magnetic resonance imaging (MRI)</td>
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<tr>
<td>Histopathology Analysis of Synovial Fluid [49]</td>
<td></td>
<td>TI weighted images (1.5T) of hand [51]</td>
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<tr>
<td></td>
<td></td>
<td>- RA MRI Scoring method</td>
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<tr>
<td></td>
<td></td>
<td>- Bone marrow edema (0-3),</td>
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<td></td>
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<td>- Bone erosions (0-3),</td>
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<td></td>
<td></td>
<td>- Synovitis (0-3)</td>
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<tr>
<td>a. Health Assessment score</td>
<td></td>
<td>e. Ultrasound</td>
</tr>
<tr>
<td>- HAQ –DI score (0-4) [50]</td>
<td>a. Color Doppler ultrasound of hand [52]</td>
<td>- Resistive index</td>
</tr>
<tr>
<td>- DAS28 score (0-10) [50]</td>
<td>- Pulsatality index</td>
<td></td>
</tr>
<tr>
<td>b. Central / Peripheral Dual energy X-ray Absorptiometry (DXA)</td>
<td>- Synovial thickness</td>
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</tr>
<tr>
<td>- Whole body BMD (g/cm²)</td>
<td></td>
<td>b. Quantitative ultrasound [53]</td>
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<tr>
<td>- Regional BMD (g/cm²)</td>
<td>- Estimated Heel BMD (g/cm²)</td>
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</tbody>
</table>
method using automated Olympus analyser (Olympus AU2700, Hamburg, Germany). The hemoglobin is slightly decreased in RA patients and it averages around 10g per dl. The WBC counts (>10,000 counts/ml) usually increase in RA patients. The advanced biomarker to diagnose the RA includes anti-cyclic citrullinated peptide (ACCP) antibody and human leukocyte antigen D (HLA-DR) related gene. An increased level of ACCP above 25 U/ml is the indicative of RA [43]. A positive HLA-DR with increased level is present in RA patients [44].

2.2 X-RAY METHOD

A standard both hand radiographs are acquired at an anterior-posterior (AP) view by an experienced x-ray imaging technologist using an x-ray machine (e.g heliphos d 500mA, Siemens, India), and film (AGFA blue sensitive film). The x-ray machine has an x-ray tube voltage of 40 kV, exposure dose of 3 mAs, and film focus distance of 1m. The developed x-ray film has been digitized using a digitizer (e.g. LASER film digitizer, Model: 2905 Array Corporation Netherlands, Europe).

2.2.1 Visual Scoring Method

Valid scoring methods are available to study the inflammatory activity in the hand joints of RA [54]. The simple manual scoring methods available to evaluate the radiograph of the hand are Larsen score and Genant/sharp score. The Larsen method is based on a global score of each joint ranges from 0 to 5. The Genant/Sharp scoring method provides a separate score for bone erosions (0 to 3.5) and joint space narrowing (0 to 4) in hand joints. The Vander Heijde scores ranged from 0 to 5 in the evaluation of erosions and scores (0 to 4) for joint space narrowing. In Wistar rat-RA model, the following scores, proposed by Pathak et al [47] are used to evaluate RA based on visual assessment of its whole-body X-ray:

- Erosions : 0-3
- Joint space narrowing : 0-3
- Joint space destruction : 0-3
Where 0-indicate normal, 1-represent mild RA, 2-show moderate RA, and 3-indicates severe RA

2.2.2 Image Processing Methods

The image processing techniques used for analyzing the hand radiograph are classified as follows: i) geometric measurement ii) segmentation and iii) feature extraction technique. In geometric measurements, JSW are measured at 1st MCP-5th MCP joints, and cortical thickness are measured at 2nd, 3rd and 4th metacarpal bone of both the hands using Materialise Interactive Medical image control system (MIMICS, Materialise, Belgium, Germany) software. In segmentation method, dual tree complex wavelet transform (DTCWT) based watershed algorithm is used for automated segmentation of hand radiograph. In feature extraction method, the gray level co-occurrence matrix (GLCM) method is used to extract the statistical texture features from the required ROI of MCP joints of the segmented image.

2.2.2.1 Geometric measurement

A standard digital hand both AP and PA view x-ray in JPG format is analysed using MIMICS software. The hand bone contours are segmented semi-automatically using thresholding, and the region growing tools available in MIMICS software. The JSW was measured from the standard PA view of both the hand x-ray. From the x-ray, it was measured at the distance from midpoint of proximal phalanx to the midpoint of metacarpal head. The JSW is semi-automatically measured in 1st MCP-5th MCP joints using 3D distance measurement tool with an accuracy of 0.01mm. The combined cortical thickness (CCT) is measured at diaphysis on 2nd, 3rd and 4th metacarpal bone using 3D distance measurement tool. In a cylindrical segment of a long bone, at 50% length of the metacarpal bone, the periosteal diameter (D) and endosteal (medullary cavity) diameter (d) are measured. From these measurements, the % CCT is calculated as follows:

\[ \% \text{CCT} = \left(\frac{D-d}{D}\right) \times 100 \] (2.1)
2.2.2.2 Image segmentation algorithm

The DTCWT based watershed algorithm is used to segment the hand from the radiograph. The detailed algorithm is given in steps as follows:

i). The input hand x-ray image is preprocessed using Gaussian filter.

ii). DTCWT is applied to the preprocessed image.

iii). The texture gradient and modulated gradient image are obtained.

iv). The total gradient image is obtained from the summing of texture and modulated gradient image.

v). Watershed transform is applied to the total gradient image.

vi). Initial segmented regions of bones in hand radiograph is obtained

vii). Region contours of segmented image superimposed on the initial input image.

viii). ROI is marked manually in MCP1-MCP5 joints of the segmented image.

ix). Features are extracted from the ROI of the segmented image using GLCM method.

The DTCWT executes a complex wavelet transforms using dual trees of filters and two diverse sub band filtering schemes for real and imaginary parts, separately have been carried out [55,56]. It possesses the features of scale and orientation sensitivity and is approximately shift invariant and provides a representation with reduced redundancy. Six sub bands are created beside six different orientations at $\pm 15^0$, $\pm 45^0$, $\pm 75^0$ for each scale level preserving the feature details of the original image [57]. The texture features were acquired by the implementation of directional median filtering to each sub band of DTCWT. The directional median filtering refers to median filtering adapted to the orientation $\theta$ of the sub band $i$. It is realized as two 1D median filters $f_{M(0+\pi/2)}$ and $f_{M\theta}$, where first
filter eliminates the double edge effect of sub bands and second filter gets rid of the noise in the sub bands as given in Equation 2.2.

\[
M_{\omega, \theta}(x_1, x_2) = \sum_{m, \theta} \left( f_m \left[ D_{\omega, \theta}(x_1, x_2) \right] \right)
\]  
\[\text{(2.2)}\]

The new sub bands are passed to the Gaussian derivative function to evaluate their gradients, after directional median filtering. Then, a modulated gradient is attained, after the texture gradient of the image has been acquired. The modulated gradient is based on the texture activity [58]. Its objective is to curb the intensity gradient in textured areas, but retain it unchanged in smooth regions.

The measure of texture activity is described by

\[
f_r(x_1, x_2) = e^{R_{\text{half}}(E_\Gamma(x_1, x_2) / (\lambda - \psi))}
\]  
\[\text{(2.3)}\]

The value of \(\lambda = 2\) and \(\psi = 7\) are two predefined parameters used for the 8-bit grayscale image [59] in equation 2.3. These values are determined by varying the range from 1 to 10 and the corresponding output images were visually inspected whether it had an optimum image segmentation or not. It was found that when the values of \(\lambda\) and \(\psi\) were 2 and 7 respectively, the obtained output image had an optimum image segmentation.

where \(E_\Gamma\) is the texture energy, \(R_{\text{half}}(\zeta)\) is half-wave rectification to suppress negative exponents as given in Equation 2.4.

\[
R_{\text{half}}(\zeta) = \begin{cases} 
0, & \text{when } \zeta < 0 \\
\zeta, & \text{when } \zeta \geq 0
\end{cases}
\]  
\[\text{(2.4)}\]

\[
E_r = \sum_{\omega, \theta} \int f_z \left( \varepsilon_k \left( \frac{M_{\omega, \theta}(x_1, x_2)}{2^z} \right) \right)
\]  
\[\text{(2.5)}\]
Where $\mathbb{E}_k$ the morphological erosion operator with structure element $k$. $k$ is a square neighborhood of nine pixels. $M_{i\theta}(x_1, x_2)$ is the subbands of the directional median filter. $f_z$ is the simple zero insertion interpolation function as specified in Equation 2.5.

The texture gradient and modulated gradient are combined together to form the modified gradient as specified in Equation 2.6.

$$G_M(x_1, x_2) = \frac{\left| \nabla F(x_1, x_2) \right|}{T_A(x_1, x_2)} + \frac{T_G(x_1, x_2)}{M_T}$$

(2.6)

Where $M_T$ is the median value of the texture gradient, $M_I$ is defined to be four times the median intensity gradient, $T_A$ represents the texture activity and $\nabla F(x_1, x_2)$ is the gradient of the original image. $T_G$ represents a single texture gradient map. The first part of the equation 2.6 represents the texture gradient and the second part represents the modulated gradient.

The watershed transformation is a technique for segmenting the images that utilize the type of region growing method based on image gradient. It efficiently merges the elements from both the discontinuity and similarity schemes of the segmentation process. The background of watershed transform has been evolved from the geography; it is a landscape or a topographic surface which is swamped by water, watershed is a ridge that separate areas drained by different river system. The two different methods for watershed transform are rain falling approach and immersion simulation. In rain falling approach, rain drops initiate at the topographic surface and reaches the catchment basin until it reaches the minimum point. A catchment basin is the area in which rain drops into the river or reservoir. The process is repeated so that different catchment basins are shaped which is being split by watershed lines. In the second method, i.e. immersion simulation, visualize that landscape being engrossed in lake with holes penetrated in the regional minima, water will go through into these holes to submerge the surface. During this process, two or more floods directed from different regional minima are combined. Hence, in
order to check the merging process, virtual dams has been built. These dams are referred to as watershed lines. The watershed transform incorporates these ideas to grayscale image processing to decipher the various segmentation problems. In watershed transform, the topographic surface is regarded as a grayscale image and height as an intensity function $f(x,y)$. The watershed algorithm discovers the catchment basin and ridge lines in the grayscale image [60].

The regions that have been over segmented are grouped in region merging process. Generally, the DTCWT followed by watershed transformation produces meaningful region segmentation. However, in order to have more reduction in the regions, region merging is used. This is achieved by if the intensity of two adjacent regions is smaller than a threshold, they will be combined and reduces the number of regions. Finally the regions contours of the segmented image are superimposed on the input hand radiograph image. The ROI is indicated in the MCP of all the fingers, manually in the final segmented image. The flowchart for image segmentation algorithm is given in Figure 2.1.

2.2.2.3 Feature extraction using GLCM method

The segmented image of whole hand is taken as an input image and the ROI is marked manually in the 1st -5th MCP joints of the segmented image. The features are extracted from the marked ROI using the GLCM method. The GLCM method is used to characterize the texture of the image based on pixel intensity distribution and its relative position in the image. It is the statistical method of extracting texture features from the second order statistics. It calculates how often a pixel with the intensity value ‘i’ occurs in a specific spatial relationship to a pixel with the value ‘j’ [61]. It means that the frequency of occurrence of gray level value ‘i’ occur either horizontally, vertically or diagonally to adjacent pixels with the value ‘j’. The GLCM matrices are constructed at a distance of $d=1, 2, 3, 4$ and in the direction of data given as $0^0, 45^0, 90^0$ and $180^0$. In the proposed approach, the features such as mean, standard deviation, variance, entropy, normalized mean, mean angle, exponential component, autocorrelation and covariance are extracted.
Figure 2.1  Flow chart of the automated image segmentation using DTCWT based watershed algorithm and feature extraction
from the ROI of the segmented image of the total studied population using the GLCM method. In the present study, nine features are considered and are described as follows: The mean is defined as the average gray level with respect to the central position. The standard deviation is a measure of dispersion or variation from the mean value. A low standard deviation represents the data points to be very close to the mean value and a high standard deviation represents the data points are spread out over the large range of values. The variance is the square of the standard deviation. The small variance indicates data points are close to the mean, whereas the large variance represents the data points are spread out from the mean. Entropy is a measure of the randomness present in the intensity distribution. It is the quantitative measure of information provided by the image. If the pixels have same gray level in the image, then minimum entropy is achieved, whereas for the pixels with uniform distribution of gray levels or histogram equalized gray level image, maximum entropy is achieved. The normalized mean is scaling the mean of the image to the normalized value. The mean angle will calculate the set of angles in degrees based on polar considerations in gray levels. The autocorrelation texture measures the linear dependency of gray levels on those of neighboring pixels. Covariance is the measure of the linear relationship between two variables. It provides the measure of correlation between the two or more sets of random variables [62].

The fundamental equations of nine features obtained from GLCM method [63] are given as follows:

i) Mean

\[
\text{mean} = \sum_i \sum_j i \cdot P(i,j) \quad (2.7)
\]

ii) Standard deviation

\[
\sigma = \left( \sum_{i=0}^{N-1} \sum_{j=0}^{N-1} (i - \mu)^2 P(i, j) \right)^{1/2} \quad (2.8)
\]
iii) Variance

\[ \text{Var} = \Sigma j P(i,j) (i-\mu)^2 \]  

(2.9)

iv) Autocorrelation

\[ \text{Auto corr} = \sum_{i=0}^{N-1} \sum_{j=0}^{N-1} p(i,j) \frac{(i-\mu)(j-\mu)}{\sigma^2} \]  

(2.10)

v) Entropy

\[ \text{Ent} = \Sigma j P(i,j) \log(P(i,j)) \]  

(2.11)

vi) Normalized mean

\[ \text{Norm mean} = \frac{1}{N} \Sigma j P(i,j) \]  

(2.12)

vii) Exponential component

\[ y = \exp[-P(i,j)] \]  

(2.13)

viii) Mean angle

\[ \bar{\alpha} = \alpha\tan2 \left( \frac{1}{n} \sum_{j=1}^{n} \sin \alpha_j, \frac{1}{n} \sum_{j=1}^{n} \cos \alpha_j \right) \]  

(2.14)

ix) Covariance

\[ C_X = \frac{1}{N} \Sigma j P(i,j) P(i,j)^T - \mu \mu^T \]  

(2.15)

2.3 RESEARCH METHODS

The research method used in the study of RA are classified as follows

i) health assessment scores (HAQ score and DAS28 score); ii) DXA-BMD; iii) Thermography; iv) MRI; and v) Ultrasound.
2.3.1 Health Assessment Score

The HAQ test is self administered by each RA patient, as suggested by Fries et al [64]. The functional ability of RA patients based on the HAQ score is divided into the following; i) score 0: no activity, ii) score 1: mild activity, iii) score 2: moderate activity, and iv) score 4: severe activity [65]. The functional categories assessed specifically by questionnaire are given as follows a.Dressing and grooming; b. Rising; c. Eating; d. Walking; e. Hygiene; f. Reach; and common daily activities. Based on these activities, the HAQ score was calculated by summing score of each activities divided by number of activities. The DAS28 score is used to measure the bone joint activity in RA patients [66]. It is based on the following: i) swollen bone joint counts (SJC) of 28 joints; ii) tender bone joint counts (TJC) of 28 joints based on subjective visual assessment score ranged from 0 to 28 [66]; iii) ESR; and iv) patient’s global assessment of general health (GH) based on a visual analog scale (1-100 mm). The score of each patient is calculated using the available online internet calculator (http://www.das-score.nl). Furthermore, it can be calculated manually using the following equation [67]:

\[
\text{DAS28} = 0.56 \times \sqrt{TJC} + 0.28 \times \sqrt{SJC} + 0.70 \times \ln(ESR) + 0.014 \times GH 
\]  

(2.16)

Based on the calculated score, the patient group is classified as follows: i) score <3.2: low RA activity; ii) score \( \leq 5.1 \): moderate RA activity; iii) score >5.1: high RA activity.

2.3.2 DXA-BMD

DXA is a modality used to quantify the BMD(g cm\(^{-2}\)) by comparing the material’s absorption of x-rays of two different energies. It is used as a diagnostic and assessment tool in early RA. It is considered as the ‘gold’ standard method in determining BMD [68]. The BMD in the forearm region is measured using peripheral DXA (Osteometer model-DTX-200, DEXA care Meditech. Inc, Hawthorn, California, USA). It includes the following BMD measurement of forearm region: i) ulna; ii) distal; iii) radius; and iv) n-ROI. The ulna BMD (U-
BMD) measurement alone is included in the study analysis. The reason for choosing ulna region in forearm is given as follows: i) The ulnar side of the wrist is often the first place of significant synovitis in the rheumatoid wrist [69]; ii) Ulna region was very much affected in RA due to the synovial expansion causing the inflammation in the ligaments of the wrist which in turn causes the ulnar translation [70]; iii) The classification of RA into the different types of disease severity at the wrist level was performed based on serial radiographs with measurements of ulnar translation [71]; iv) Dorsal dislocation of ulna of more than 6 months was an additional risk factors of RA[72]. The accuracy and sensitivity of the device are reported as 95% and 99% respectively [73].

2.3.3 Thermography

Thermal imaging has been utilized preclinically as a tool for evaluation of inflammation and arthritis. It is a non-invasive, non-contact type, non-ionizing method for the diagnosis of RA. It provides a functional imaging method for analyzing physiological function related to body temperature. It provides a thermal pattern of the skin temperature in the area of observation. Infrared thermography provides directly a digitized output called a ‘thermogram’. It is defined as a two dimensional radiance function \( r(x, y) \), where ‘x’ and ‘y’ denote spatial coordinates and the value of ‘r’ at any point is proportional to the radiance [37].

2.3.3.1 Thermal image acquisition and analysis

A hand held thermal camera (ThermaCAM-T400, FLIR version 1.2, Sweden) is used at both the posterior-anterior(PA) view and AP view in order to image the hand region. The Thermal camera T400 utilizes the 320x420 thermal element focal plane array (FPA) uncooled microbolometer detector system with a minimum focusable distance of 0.4m to infinity. The camera could measure the temperature range of \(-20^\circ \text{C}\) to \(1200^\circ \text{C}\) to an accuracy of \(\pm 2^\circ \text{C}\) with a thermal sensitivity of \(0.05^\circ \text{C}\). Prior to image acquisition, a subject is asked to sit with his/her
hand exposed for 10 minutes in a temperature controlled room at 20°C, with the humidity of 45-50%. The thermal camera is positioned at a distance of 1.0m to obtain a thermogram of the hand in the neutral position. The image is analyzed by using FLIR software version 1.2, and is further processed with MATLAB version 7.1.

A total of 28 index joints per subject which includes 10 distal interphalangeal joints (DIP), 8 PIP and 10 MCP are evaluated. The skin temperature is measured using an area tool in the defined square region of interest (ROI) of size 8x8 mm positioned over the DIP, PIP, and MCP joints of the hand digits 1-5 using the FLIR software. The software has determined the minimum, maximum and average temperature for the operator defined ROI. The localization of ROI of each finger joints such as DIP, PIP, and MCP has been semi-automatically selected. The skin surface temperature is measured over the dorsal midpoint of each joint and the ventral side (palm region).

In small animal RA model study, thermal images of both fore limbs and hind limbs of wistar rats are acquired right from the zeroth day in a PA view without inducing anesthetic agents. From the images, the skin temperatures are measured in the fore limb and hind limb as mentioned above. The mean absolute temperature is determined at the mid-point of the phalangeal region of the fore limb and hind limbs of wistar rat.

2.3.3.2 Temperature indices

The heat distribution index (HDI) reflects the pattern and the spread of temperature over joints. The HDI is defined as the twice the standard deviation of all the temperatures within the ROI of the MCP joint [74]. Thermographic index (TI) describes the peak temperature of the joint and the area of each temperature band. It is used for quantitative evaluation of thermal changes in the ROI. The TI is calculated using the following formula [75].
\[ TI = \left( \sum \Delta T \times a \right) / A \] (2.17)

Where \( \Delta T \) represents the difference between the mean temperature over the monitored area and the temperature of isotherm and ‘a’ indicates the area occupied by the isotherm (sq.cm) and ‘A’ represents the total area of thermogram (sq.cm). The diagnostic values of TI value are given as follows: as i) 2-normal; ii) 4-OA; and iii) >4-RA[76].

2.3.3.3 Thermal image segmentation

Automated thermal image segmentation is performed by implementing k-means algorithm in hand thermal images of both dorsal and ventral views. K-means is an unsupervised clustering algorithm which classifies or group the data based on features into k number of groups. The grouping is done by minimizing the sum of squares of distance between data and corresponding cluster centroids. The flowchart for automated image segmentation using k-means algorithm is given in Figure 2.2.

K-means algorithm [77] as follows:

i) Input image is the hand thermal image taken in dorsal/ventral view

ii) The RGB image is converted to HSV image.

The reason for conversion of RGB to HSV conversion is as follows: i) RGB defines color in terms of a combination of primary colors, whereas HSV describes it using more familiar comparisons such as color, vibrancy and brightness; ii) Further HSV describes color similarly to how the human eye tends to perceive it.

iii) Choose three (k) classes and assign them as initial centroids.

Class 1: cluster 1; Class 2: cluster 2; Class 3: cluster 3

iv) Find the distance between the centroid and each pixel of the input.
v) Cluster them according to the minimum distance.

- Cluster 1- to separate hot spot region
- Cluster 2- to separate background region
- Cluster 3- to separate other than hot spot regions as well as background.

vi) Update the new centroid, which is the average of all points in the cluster.

vii) Do Steps 2 to 4 until no points, switches to the new cluster. After some ‘n’ Iterations.

In Wistar rat-RA model, the thermal image of the limb is analyzed using the following image processing techniques viz., a) Robert edge detector; b) Canny edge detector; and c) Adaptive thresholding technique. The flowchart for automated image segmentation of fore limb and hind limb of wistar rat-RA model using adaptive thresholding method is given in Figure 2.3.

a) Robert edge detector

It performed simple 2D-Spatial gradient measurement on an image [78], and the algorithm used is given as follows:

i). Apply Robert’s operator on a thermal image;

ii). Conversion of RGB to gray

iii). Apply median filter to gray image to remove noise

iv). Find the gradient magnitude and replace the original pixel by the magnitude;

v). Convolve the mask on the entire image;
vi). Convolved image is an edge detected image;

b) Canny edge detector

It used multi-stage algorithm, which is given as follows, to detect a wide range of edges in images [78]:

i). Smoothening the original image

ii). Conversion of RGB to gray

iii). Apply median filter to gray image to remove noise

iv). Finding the intensity gradient of the image.

v). Apply non maxima suppression.

vi). Apply thresholding and track the edges

c) Adaptive thresholding [78]

The algorithm used is given as follows:

i). Input thermal images of the hind limb and fore limb of Wistar rat

ii). Conversion of RGB to gray for preprocessing

iii). Apply median filter to the gray image to remove noise.

iv). Convolve the image with median

v). Subtract the original image with convolved image

vi). Threshold the difference image with c=8

‘c’ is a threshold constant, it can be varied from 0 to 255.
Figure 2.2  Flow chart of automated hand thermal image segmentation using k-means algorithm

Figure 2.3  Flowchart of automated segmentation of fore limb and hind limb of wistar rat- RA model using adaptive thresholding
2.3.3.4 Feature extraction

The feature extraction technique is implemented over the segmented output image to extract the intensity features and statistical texture features. The features such as mean, standard deviation, smoothness, entropy, kurtosis, skewness, variance, contrast, correlation, and energy are extracted in the segmented output image using GLCM method. The mean is the average intensity value of the hot spot regions. The standard deviation is a measure of dispersion or variation from the mean value. A low standard deviation represents the data points to be very close to the mean value and a high standard deviation represents the data points are spread out over the large range of values. Entropy is the measure of randomness that can be used to characterize the texture of the image. It is the quantitative measure of information provided by the image. If the pixels have same gray level in the image, then minimum entropy is achieved, whereas for the pixels with uniform distribution of gray levels or histogram equalized gray level image, maximum entropy is achieved.

The skewness is the measure of the asymmetrical distribution around its mean. The symmetric distribution has zero skewness and has equal values for mean, median and mode. If the skewness is positive, then the data are positive skewed or skewed right, meaning that the right tail of the distribution longer than the left with mean is greater than the median and the mode is less than the median. If the skewness is negative, the data are negatively skewed, meaning that the left tail of the distribution is longer than the right with the mean is less than the median and the mode is greater than the median.

The kurtosis refers to the measure of the peak of the array of intensity distribution. Similar to skewness, kurtosis is a descriptor of the shape of the probability distribution. There are three different ways of interpretations of kurtosis such as mesokurtic distribution, leptokurtic distribution and platykurtic distribution. A zero excess kurtosis indicates the mesokurtic distribution which has a normal peak around the mean. A positive excess kurtosis represents the leptokurtic distribution
which has a more acute peak around the mean. A negative excess kurtosis shows the platykurtic distribution which has a lower, wider peak around the mean [79].

The variance is the square of the standard deviation. The small variance indicates data points are close to the mean, whereas the large variance represents the data points are spread out from the mean. The contrast is the measure of the intensity contrast between the pixel and its neighbor over the image. The correlation texture measures the linear dependency of gray levels on those of neighboring pixels. Energy provides the sum of the squared elements in the GLCM. Energy measures the textural uniformity that means pixel pairs repetitions. Energy is also the features that measure the smoothness of the image. For more uniformly distributed pixels, less smooth the regions and have low energy. In case of the non uniform distributed region, the smoothness and energy are high. Hence, in case of RA patients, due to uneven distribution of temperature in hand regions, the energy obtained was higher compared to the normal [80].

2.3.4 MRI

Magnetic resonance imaging (MRI) could directly detect the early findings of disease like bone marrow edema, bone erosions and synovitis in RA [51]. It has an advantage over other imaging modalities like plain X-ray and CT, in its ability to image the soft tissues and fluid within the joints. It has been used in clinical applications for the detection of erosions and prediction of prognosis in RA patients, which could influence management decisions in early disease. Several researchers have developed an automated method using fuzzy c-means algorithm, otsu’s thresholding for segmentation and quantification of inflammatory tissue of hand in RA patients [81,82].

2.3.5 Ultrasound

Ultrasound (US) could be used as a valuable tool in rheumatology for a wide spectrum of indications including inflammatory arthritis, tendon pathology, disease activity monitoring, and disease progression monitoring [83]. It is possible
to detect the morphological changes like synovial thickness and bone erosions with grayscale US [84], but the blood flow in synovium and surrounding tissue is demonstrated using color Doppler US [39]. Unlike the conventional US, color Doppler US depicts the real time representation of arterial and venous blood flow and velocity in MCP joints of the hand. The use of color Doppler studies provides a measure of neovascularisation within the synovial lining of joints and tendons which is not available in other imaging modalities [85].

2.4 BIOPSY/AUTOPSY METHOD

2.4.1 Histopathological Analysis of Synovial Fluid

A specimen of the RA affected joint is obtained through closed needle biopsy or arthroscopic guided biopsy. Then, it is stained with hematoxylin and eosin. The histological evaluation of synovial tissue inflammation in RA confirms the hyperplasia in the lining layer, cellular infiltration with lymphocytes, macrophages, granulomas, pannus formation and plasma cells [86]. In Wistar rat-RA model, the rats are sacrificed on the 30th day and their forepaws and hind paws are fixed as described below. The ankle joints are separated and kept at 10% neutral buffered formalin for 24 hrs prior to placement in surgipath decalcifier for approximately one week. Paws are decalcified with a solution containing HCL and 0.1M EDTA. After completion of the decalcification process, the ankle joints are transacted into two equal halves in longitudinal plane and are embedded in paraffin, sectioned, and stained with haematoxylin-eosin. The histological scoring system is done for the following skeletal sites: tibio-talar, talus-calcaneal, Mid-foot joints and mid-finger joints. In Wistar rat-RA model, the published histological scoring system is used to measure the following variables semi-quantitatively with score ranges from 0-3: i) enlargement of synovial lining cell layer i) synovial hyperplasia; ii) inflammatory infiltrate; iii) synovial vascularity; iv) cartilage erosions; v) bone erosions; and vi) pannus formation [49,87,88]. The score 0 indicates the absence of particular variable; score 1 represents mild of particular variable; score 2 depicts moderate of particular variable; score 3 illustrates the severity of particular variable.
2.5 CLASSIFIER: FEED FORWARD BACKPROPAGATION NETWORK

Feed forward neural networks are one class of neural network used to solve complex problems by modeling complex input-output relationships [89]. The back propagation learning algorithm is a widely used method for feed forward neural networks in many medical applications[90]. The feed forward back propagation network consists of three layers namely input layer, hidden layer and output layer. It is trained by adjusting the weights in order to perform perfect classification using the back propagation learning algorithm. The feed forward back propagation network make use of binary sigmoid activation function to scale the hidden layer and output layer of neural network. The input features are normalized between 0 and 1 before feeding to the network. The desired output is indicated as ‘0’ for normal and ‘1’ for abnormal. The classification process is categorized into four phase as follows: i) training phase ii) testing phase iii) validation phase and iv) resultant phase. By using the input patterns, learning repetitions is performed with different training and validation data sets. The ROC plots are used to determine the accuracy of the classification method obtained from the network.