Intravoxel Incoherent Motion (IVIM) and Multi-parametric MRI Analysis for Chemotherapy Response Evaluation in Bone Tumor

Esha Baidya Kayal

CENTRE FOR BIOMEDICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY DELHI

OCTOBER 2020

© Indian Institute of Technology Delhi (IITD), New Delhi, 2020

Intravoxel Incoherent Motion (IVIM) and Multi-parametric MRI Analysis for Chemotherapy Response Evaluation in Bone Tumor

by

Esha Baidya Kayal

Centre for Biomedical Engineering

Submitted

In fulfillment of the requirements of the degree of Doctor of Philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

OCTOBER 2020

Dedicated

to my Parents

CERTIFICATE

This is to certify that the thesis entitled "**Intravoxel Incoherent Motion (IVIM) and Multi-parametric MRI analysis for Chemotherapy Response Evaluation in Bone Tumor**", being submitted by **Mrs. Esha Baidya Kayal**, to the Indian Institute of Technology Delhi, for the award of '**Doctor of Philosophy**' in Centre for Biomedical Engineering is a record of the bonafide research work carried out by her under our supervision and guidance. She has fulfilled the requirements for submission of this thesis, which to the best of our knowledge has reached the requisite standard. The materials contained in the thesis has not been submitted in part or full to any other University or Institute for the award of any other degree or diploma.

Dr. Amit Mehndiratta
Associate Professor
Centre for Biomedical Engineering
Indian Institute of Technology, Delhi

Dr. Devasenathipathy Kandasamy
Additional Professor
Department of Radio Diagnosis
All India Institute of Medical Science, Delhi

Date: 16/10/2020
New Delhi

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my chief supervisor Dr. Amit Mehndiratta. He has been a true mentor throughout my PhD with his knowledge, continuous guidance, critical comments, suggestions and encouragement. I should not forget to mention the constant work-schedules and the non-negotiable time-lines of the deliverables, which motivated me the most to work hard and bring out the best possible output. Honestly expressing, Dr. Amit has redefined my capabilities and I will be ever indebted to him for that. He is my guru in the true spirit.

I would like to thank my co-supervisor Dr. Devasenathipathy Kandasamy for enriching me with his clinical knowledge throughout my PhD. I must mention his guidance, thoughtful suggestions and encouragement in all stages of my PhD and he handled all my endless queries regarding radiological interpretation and explanation of pathology with remarkable patience.

Next, I must thank my clinical research team at AIIMS, Delhi. Dr. Sameer Bakhshi facilitated the patient recruitment at his OPD in IRCH, AIIMS, Delhi. He provided me the important clinical knowledge about osteosarcoma cancer biology and chemotherapy treatment management and useful suggestions and advice whenever it was required. The patience and care with which he deals with his patients is worth witnessing. My attitude towards life has changed positively after being in his association.

I would also like to express my deep gratitude to Dr. Raju Sharma, Department of Radiology, AIIMS, Delhi for his succinct suggestions, advice and encouragement all through my PhD.

I am grateful to my respected research committee members at IIT Delhi for their valuable feedback and suggestions time to time. I would like to express my sincere gratitude to Dr Kedar Khare for his critical analysis, suggestions and guidance in the methodological development of my thesis. I must thank Dr. Anup Singh for his constant support and encouragement. His thoughtful suggestions throughout my PhD made my project better. Dr. Veena Koul as the chairperson of my research committee provided me valuable advice and encouragement throughout. I would like to express my thanks to all the faculty members and office staff in our center.

I must thank the nursing team and the support staff at medical oncology OPD in IRCH, AIIMS, Delhi, for their sincere cooperation during patient recruitment and the MRI technician

team under Department of Radiology, AIIMS, Delhi, for their help during MRI acquisition. There are many days the team worked extra hours to get my cases done, despite the huge patient load.

I would like to thank the participating patients who provided the most important contribution to carry out this research work. Inspite of their illness and painful pathological condition with such a morbid tumor like osteosarcoma, they gave their consent for participation and cooperated during MRI acquisition. Without them this research would not be possible. Their sufferings have made me humble and realize the blessings I have in life. and I sincerely pray for their cure and comfort.

I am thankful to all my lab-mates, especially Neha, Anirban, Ayan, Snekha, Rafeek, Dinil, Rupsha, Sandeep, Dharmesh, Archana, Virender, Debashish, Shruti, Rakshit, Sameer, Banasmita, and Neha for their encouragement and comradeship and making this journey memorable and enjoyable. I would also express my acknowledgment and affection to the intern team, Abhimanyu, Rishabh, Akshay, Kabir, Sneha, and Nikhil for their valuable input in data processing and implementation of various stages of segmentation algorithms. Mentoring them was a good learning experience for me.

I acknowledge and thank the Ministry of Human Resource Development, Government of India for providing the research fellowship funds required for my PhD. I would also like to thank DBT, INSA, CSIR, ICMR and IITD research scholar fund for providing me travel grant award for attending international conferences.

Special thanks and gratitude to my parents and elder sister for their love and blessing. Their support is the backbone of my success. I am grateful to my friend, Thilaka for her support and encouragement and being with me at all ups and downs. This list is incomplete if I do not mention Sulagna, Souvik, Sudipta and Jeeva, who constantly encouraged me throughout the course of my PhD. Last but not the least, I would like to thank my husband for his unstinting belief in me. He always pushes limits for me and is the only reason why I joined IIT. Words are not enough to thank him for all the patience and support he has given me always. Such comfortable burning of the midnight oil would not have been possible without his understanding.

Being a part of MedImg research team at CBME is a great experience and my PhD in this eminent Institution like IIT Delhi has been a great journey. I shall be thankful to almighty forever for giving me such an opportunity and providing me enough luck to successfully and timely complete my PhD research work.

(Esha Baidya Kayal)

ABSTRACT

Osteosarcoma is the most common bone sarcoma and the third most common malignancy in children and adolescents with high morbidity and mortality. Early evaluation of chemotherapy response may help to prevent the patients from undergoing ineffective chemotherapy regimen, reducing side-effects, saving treatment time, cost and may improve patient management through personalized therapeutic options. The goal of this PhD thesis was to investigate the role of non-invasive imaging-based markers for monitoring and evaluating early therapeutic response in patients with osteosarcoma receiving neoadjuvant chemotherapy using Intravoxel incoherent motion (IVIM) and mutli-parametric MRI analysis. Existing challenges in literature regarding anti-cancer therapeutic response evaluation in bone tumor were attempted to be addressed in this PhD research work.

The first objective of this thesis was to develop the methodology for reliable and reproducible IVIM parameter estimation as the existing widely used IVIM analysis methodologies, viz bi-exponential (BE) model and segmented BE techniques, evaluate IVIM parameters at each voxel independently, overlooking the spatial context in tissue which may lead to unreliable noisy solutions. To achieve this goal, two gradient-based adaptive penalty functions, Total Variation (TV) and Huber penalty function (HPF) were incorporated into the non-linear least-square (NLLS) optimization of standard BE model and two novel IVIM analysis methodologies 1) BE model with Total Variation Penalty function (BE+TV), and 2) BE model with Huber Penalty function (BE+HPF) were developed. Proposed BE+TV and BE+HPF methods, adaptively adjust the NNLS error and reduce the non-physiological spatial inhomogeneity and noise in parameter estimation by using TV/HPF penalty reduction at each iteration of NNLS optimization to produce reliable

and reproducible parametric images. Experimental results using simulation and empirical clinical datasets showed quantitatively and qualitatively improved IVIM parameter estimation by proposed BE+TV and BE+HPF methodologies than the existing BE and segmented BE techniques. The performance of the two developed methodologies were similar.

The second objective was to assess the potential of quantitative IVIM analysis for characterizing and evaluating early chemotherapeutic response in patients with osteosarcoma. IVIM parameters such as diffusion coefficient (*D*), perfusion coefficient (*D**) and perfusion fraction (*f*) were evaluated using state of the art IVIM analysis methodology BE+TV along with apparent diffusion coefficient (*ADC*) and histogram analysis was performed for these parameters. Experimental results demonstrated the potential of IVIM analysis for evaluating chemotherapy response in osteosarcoma with correlation to both the radiological and the histopathological response evaluation measurements as the reference standards. IVIM perfusion-related parameters (*D*, f*) and their histogram parameters - standard-deviation, energy and entropy were effective to be used as surrogate markers for characterizing heterogeneity in tumor micro-vasculature and its changes during chemotherapy in osteosarcoma.

The third objective was to develop a robust and novel automated methodology to delineate, visualize and quantify the proportion of necrosis and viable tissue present within the tumor and eventually evaluate the chemotherapeutic response using multi-parametric MRI. To achieve this goal, Simple linear iterative clustering supervoxel (SLICs) and Otsu multi-thresholding (Mth) were combined to develop the proposed SLICs+MTh methodology; while the former technique clusters the voxels with similar intensity levels in close proximity and latter technique selects and combines the clusters to sub-segment the targeted pathological region within the tumor. The proposed methodology SLICs+MTh is non-invasive and imaging based and uses diffusion

weighted image (DWI), T2 weighted fat-saturated image and *ADC* parametric map. SLICs+MTh was applied to the tumor before and after the chemotherapy regimen and it produced reliable approximation of amount of macro-necrosis and viable tumor volume in osteosarcoma which was in satisfactory agreement with the estimated histopathological necrosis after surgery.

The fourth objective was to assess the efficacy of multi-parametric 3D statistical texture analysis in characterizing tumor microstructure and its changes during chemotherapy in osteosarcoma and evaluating early therapeutic response. To meet the purpose, textural features based on Gray-level co-occurrence matrix (GLCM), Neighborhood gray-tone difference matrix (NGTDM) and Run length matrix (RLM) were evaluated on T1W, T2W & DWI images and *ADC*, *D*, *D** & *f* parametric maps. A linear discriminant analysis was performed to find the potential surrogate markers for tumor aggressiveness and responsiveness to chemotherapy. NGTDM features coarseness, busyness and strength for *D, D** & *f* and T1W, T2W images, acquired even before start of the chemotherapy; were found to be useful markers for predicting tumor aggressiveness and prognosis. GLCM features contrast, correlation; NGTDM features contrast, complexity and RLM features short run low gray-level emphasis; for *D, D** & *f* and the T2W image, were found to be effective markers for chemotherapy response early in the course of treatment.

This PhD research work developed methodologies for reliable quantitative IVIM parameter estimation and identified imaging-based markers for monitoring and evaluating chemotherapeutic response in osteosarcoma by applying various analyses using IVIM and multi-parametric MRI. The research findings of this thesis might help the oncologist and radiologists in performing early prediction and evaluation of the chemotherapy response in patients with osteosarcoma and therefore might be beneficial for the patients by enabling personalized treatment regime.

सार

ओस्टियोसारकोमा सबसे आम अश्थी कैंसर है और तीसरी सबसे आम कैंसर है, जो बच्चो और किशोरों में उच्च-रुग्णता और मृत्यु-दर के साथ पाए जाते हे। कीमोथेरेपी की प्रारंभिक प्रतिक्रिया मूल्यांकन, अप्रभावी कीमोथेरेपी के दौर से गुजर रहे रोगियों में कीमोथेरेपी की पार्श्व-प्रभाव को कम करने में मदद कर सकती है, उपचार समय और लागत की बचत हो सकती है, और व्यक्तिगत चिकित्सीय विकल्प के माध्यम से रोगी प्रबंधन में सुधार हो सकता है। इस पीएचडी शोधलेख का लक्ष्य था, ऑस्टियोसारकोमा रोगियों में कीमोथेरेपी उपचार के दौरान प्रारंभिक चिकित्सीय प्रतिक्रिया और निगरानी के लिए इंट्रावॉक्सेल इनकोहेरेंट मोशन (आई.वी.आई.एम.) और मल्टी-पैरामीट्रिक एमआरआई द्वारा उपलब्ध गैर-हस्तक्षेप, इमेजिंग-आधारित मार्कर की भूमिका का मूल्यांकन करना। इस अनुसंधान कार्य में, अश्थी कैंसर में चिकित्सीय प्रतिक्रिया मूल्यांकन करने के लिए वैज्ञानिक साहित्य में मौजूदा चुनौतियों को संबोधित करने का प्रयास किया गया है।

इस शोधलेख का पहला उद्देश्य था, विश्वसनीय और पुनरवर्तनीय आई.वी.आई.एम. मापदंडों का अनुमान करने के लिए कार्यप्रणाली विकसित करना; क्यूंकि मौजूदा व्यापक रूप से उपयोग किया गया आई.वी.आई.एम. विश्लेषण के तरीके, यानी बीएक्सपोनेंशल (बी.ई.) मॉडल और खंडित बी.ई. तरीकों, स्थानिक संदर्भ की अनदेखी करते हुए प्रत्येक वोक्सेल में स्वतंत्र रूप से आई.वी.आई.एम. मापदंडों का मूल्यांकन करते है, जिससे अविश्वसनीय और ग़लत मूल्यांकन हो सकता है। इस लक्ष्य को प्राप्त करने के लिए, दो ग्रेडिएंट-आधारित अनुकूली पेनल्टी फंक्शन, टोटल वेरिएशन (टी.वी.) और हूबर पेनल्टी फंक्शनो (एच.प.फ.) को मानक बी.ई. मॉडल का नॉन-लीनियर-लीस्ट-स्क्वायर (न.ल.ल.स.) अनुकूलन प्रक्रिया मे शामिल किये गए थे और दो नई आई.वी.आई.एम.

विश्लेषण के विधियाँ 1) बी.ई. मॉडल के साथ टोटल वेरिएशन पेनल्टी फ़ंक्शन (बी.ई.+टी.वी.), और 2) ह्यूबर पेनल्टी फ़ंक्शन के साथ बी.ई. मॉडल (बी.ई.+एच.प.फ.) विकसित किए गए थे। प्रस्तावित बी.ई.+टी.वी. और बी.ई.+एच.प.फ. विधियाँ, विश्वसनीय और पुनरवर्तनीय आई.वी.आई.एम. मापदंडों का अनुमान के लिए टी.वी./एच.प.फ. पेनल्टी फ़ंक्शन का उपयोग द्वारा आई.वी.आई.एम. मापदंडों के मूल्यांकन के दौरान न.ल.ल.स. प्रक्रिया में पाए गए गलत समाधानों को अनुकूलता से समायोजित किये थे और गैर-शारीरिक स्थानिक इनहोमोजेनिटी को कम किया था. कंप्यूटर अनुकरण डेटासेट और नैदानिक डेटासेट द्वारा प्रयोगात्मक परिणाम दर्शाता है कि प्रस्तावित बी.ई.+टी.वी. और बी.ई.+एच.प.फ. विधियाँ मौजूदा बी.ई. और खंडित बी.ई. तकनीकों की तुलना में मात्रात्मक और गुणात्मक रूप से बेहतर आई.वी.आई.एम. मापदंडों का अनुमान किया था। दो विकसित पद्धतियों का प्रदर्शन लगभग एक जैसा था।

दूसरा उद्देश्य था, ओस्टियोसारकोमा के रोगियों में प्रारंभिक कीमोथेरेपी संबंधी प्रतिक्रिया का मूल्यांकन करने के लिए मात्रात्मक आई.वी.आई.एम. विश्लेषण की क्षमता का आकलन करना। अत्याधुनिक आई.वी.आई.एम. विश्लेषण पद्धति बी.ई.+टी.वी. का उपयोग करके आई.वी.आई.एम. मापदंडों जैसे कि डिफूशन गुणांक (*डी*), परफ्यूसन गुणांक (*डी**) और परफ्यूसन अंश (*एॅफ़*) और साथ में अपरेंट डिफूशन गुणक (*ए.डी.सी.*) का मूल्यांकन किया गया था और इन मापदंडों के लिए आयतचित्र विश्लेषण किया गया था। प्रयोगात्मक परिणामों ने संदर्भ मानकों के रूप में विकिरण-चिकित्सात्मक प्रतिक्रिया और ऊतकविकृति परीक्षालब्ध प्रतिक्रिया, दोनों से सहसंबंध कीमोथेरेपी प्रतिक्रिया के मूल्यांकन के लिए आई.वी.आई.एम. विश्लेषण की क्षमता का प्रदर्शन किया गया है। कीमोथेरेपी के दौरान ऑस्टियोसारकोमा में सूक्ष्म- वाहिका और इसके परिवर्तनों में विषमता को चिह्नित करने के लिए मार्कर के रूप में आई.वी.आई.एम. परफ्यूसन से संबंधित मापदंडों (*डी*,*

ऍफ़) और उनके आयतचित्र मापदंडों - स्टैण्डर्ड-डेविएशन, एनर्जी और एन्ट्रापी का उपयोग प्रभावी पाए गए।

तीसरा उद्देश्य एक मजबूत और नई स्वचालित पद्धति का विकास करना था, मल्टी-पैरामीट्रिक एम.आर.आई. मे जिसके उपयोग से ट्यूमर के भीतर मौजूद परिगलन और कैंसर ऊतक के अनुपात और अंततः कीमोथेरेपी प्रतिक्रिया का मूल्यांकन किया जा सकता था। इस लक्ष्य को पाने के लिये सिंपल लीनियर इटेरेटिव क्लस्टरिंग सुपरवॉक्सेल (एस.एल.आई.सि.एस.) और ओट्सु मल्टी-थ्रेसहोल्डिंग (एम.टी.एच.) पद्धतियों को संयुक्त किया गया और प्रस्तावित एस.एल.आई.सि.एस.+एम.टी.एच. कार्यप्रणाली को विकसित किया गया; जबकि पूर्व तकनीक समीप और करीब इंटेंसिटी वाले वोक्सलो को संग्रह करता है और बाद की तकनीक ट्यूमर के भीतर लक्षित रोगात्मक क्षेत्रों को उप-खंडित करने के लिए चुनिंदा क्लस्टर समूहों को जोड़ता है। प्रस्तावित कार्यप्रणाली एस.एल.आई.सि.एस.+एम.टी.एच. गैर-हस्तक्षेप और प्रतिकृति-आधारित है और डिफूशन-वेटेड, टी2-वेटेड फैट-सैचुरेटेड एमआरआई और *ए.डी.सी.* मापदंड प्रतिकृति का उपयोग करती है। एस.एल.आई.सि.एस.+एम.टी.एच. कार्यप्रणाली कीमोथेरेपी के पहले और बाद में ट्यूमर पर लागू किया गया था और यह ओस्टियोसारकोमा में स्थूल-परिगलन और कैंसर ऊतक की मात्रा का विश्वसनीय अनुमान लगाया था जो शल्यचिकित्सा के बाद अनुमानित ऊतकविकृति परीक्षालब्ध परिगलन के साथ संतोषजनक सन्निपतन में था।

चौथा उद्देश्य ओस्टियोसारकोमा में कीमोथेरेपी के दौरान ट्यूमर माइक्रोस्ट्रक्चर और इसके परिवर्तनों को चिह्नित और प्रारंभिक चिकित्सीय प्रतिक्रिया का मूल्यांकन करने में मल्टी-पैरामीट्रिक 3डी टेक्सचर विश्लेषण की प्रभावकारिता का आकलन करना था। उद्देश्य को पूरा करने के लिए, टी1-वेटेड, टी2-वेटेड और डिफूशन-वेटेड एम.आर.आई., और *ए.डी.सी.,* डी, डी* और *ऍफ़* मापदंडों की

प्रतिकृति पर ग्रे-लेवल को-अकरंस मैट्रिक्स (जि.ल.सी.एम.), नेबरहुड ग्रे-टोन डिफरेंस मैट्रिक्स (न.जी.टी.डी.एम.) और रन लेंथ मैट्रिक्स (र.ल.म.) का मूल्यांकन किया गया था। ट्यूमर की आक्रामकता और कीमोथेरेपी के प्रति प्रतिक्रिया के लिए संभावित मार्कर को खोजने के लिए लीनियर डिस्क्रिमिनन्त विश्लेषण किया गया था। कीमोथेरेपी की शुरुआत से पहले ट्यूमर की आक्रामकता और रोग का निदान की पूर्वानुमान करने के लिए; डी, डी* और *ऍफ़* मापदंडों की प्रतिकृति और टी1-वेटेड, टी2-वेटेड एम.आर.आई. पर मूल्यांकित न.जी.टी.डी.एम. विशेषताएं करसेनेस्स, बुसिनेस्स और स्ट्रेंथ उपयोगी मार्कर पाए गए। उपचार के प्रारंभिक चरण पर कीमोथेरेपी प्रतिक्रिया के लिए; डी, डी* और *ऍफ़* मापदंडों की प्रतिकृति और टी2-वेटेड एम.आर.आई. पर मूल्यांकित जि.ल.सी.एम. विशेषताएं कंट्रास्ट, करेलशन; न.जी.टी.डी.एम. विशेषताएं कंट्रास्ट, कम्प्लेक्सिटी; र.ल.म. विशेषता शार्ट-रन लौ-ग्रे-लेवल एम्फेसिस प्रभावी मार्कर पाए गए।

यह पीएचडी शोध कार्य विश्वसनीय मात्रात्मक आई.वी.आई.एम. मापदंडों का अनुमान के लिए कार्यप्रणाली विकसित किया हैं और ओस्टियोसारकोमा में कीमोथैरेपि की निगरानी और प्रतिक्रिया मूल्यांकन के लिए आई.वी.आई.एम. और मल्टी-पैरामीट्रिक एम.आर.आई. का उपयोग करके और विभिन्न विश्लेषणों को लागू करके प्रतिकृति-आधारित मार्कर समूह का आकलन और खोज की हैं। इस शोधलेख के निष्कर्षों से कैंसर-चिकित्सकों और विकिरण-चिकित्सकों को ओस्टियोसारकोमा के रोगियों में कीमोथेरेपी प्रतिक्रिया की पूर्वानुमान और प्रारंभिक मूल्यांकन प्रदर्शन करने में मदद मिल सकती है और इस रूप से व्यक्तिगत उपचार व्यवस्था को सक्षम करके रोगियों के लिए फायदेमंद हो सकती है।

CONTENTS

Chapter 4: Non-invasive Chemotherapeutic Response Evaluation of Osteosarcoma using Multi-parametric MRI

Chapter 5: Texture analysis for Non-invasive Tumor Detection and Chemotherapeutic Response Evaluation in Osteosarcoma

LIST OF FIGURES

at larger b values (>200 sec/mm^2), the slope (B) of signal attenuation is less steep, which is more reflective of tissue diffusivity (This illustration was adapted from the article by Koh et al. (94)).

Figure 1.8: Imaging techniques providing information about tumor biology and microenvironment. Illustration was reused from the article by García-Figueiras et al. (192) (license: https://creativecommons.org/licenses/by/4.0/).

Figure 1.9: Patient demographic information and tumor characteristics.

Figure 2.1: The reference parametric images for A) Diffusion coefficient (*D*), B) Perfusion coefficient (*D**) and C) Perfusion fraction (*f*) for Simulation-I, II & III respectively. I – XV) Estimated parameter maps for *D*, XVI – XXX) estimated parameter maps for *D** and XXXI – XLV) estimated parameter maps for *f* by five methods at three SNR_{S0} of 60, 25 and 15.

Figure 2.2: Estimated parameter maps for A) Diffusion Coefficient (*D*), B) Perfusion Coefficient (*D**) and C) Perfusion Fraction (*f*) for Simulation-IV. I-XV) Estimated parameter maps for *D*, XVI-XXX) estimated parameter maps for *D** and XXXI-XLV) estimated parameter maps for *f* by three methods at three SNR_{S0} of 60, 25 and 15.

Figure 2.3: a. DWI (b = 800 s/mm^2) of one representative patient (M, 15 yrs) with OS in right distal femur, red circle shows the tumor; i – v) Diffusion coefficient (*D*); vi– x) Perfusion coefficient (D^*), xi – xv) Perfusion fraction (*f*) estimated with five analysis methods.
b. DWI (b = 800 s/mm^2) of one representative patient (F, 35 yrs) with ESFT in left-upper anterior chest wall, red circle shows the tumor; xvi – xx) Diffusion coefficient (*D*); xxi– xxv) Perfusion coefficient (D^*), xxvi – xxx) Perfusion fraction (*f*) estimated with five analysis methods.
Parametric images with BE+TV and BE+HPF are showing comparatively less image noise.

Figure 2.4: a, f) DWI image (b = 800 s/mm^2) of a representative patient (M, 15 years) with Osteosarcoma (same patient in Figure 2.3.a); b, g) *ADC*; c, h) Diffusion coefficient (*D*); d, i) Perfusion coefficient (*D**); e, j) Perfusion fraction (*f*). a – e) Baseline; f – j) Follow-up after 1 cycle of chemotherapy. Figures shows increase in *ADC*, *D* and reduction in D* in tumor post chemotherapy.
k, p) DWI image (b = 800 sec/mm^2) of a representative patient (F, 35 years) with Ewing's Sarcoma (same patient in Figure 2.3.b); l, q) *ADC*; m, r) Diffusion coefficient (*D*); n, s) Perfusion coefficient

(*D**); o, t) Perfusion fraction (*f*). k – o) Baseline; p – t) Follow-up after 2 cycles of chemotherapy. Figures show increase in *ADC*, *D* and *f* in tumor post chemotherapy.

Figure 2.5: Data fitting curves in tumor and healthy tissue ROIs by five IVIM analysis methods 1) BE, 2) BESeg-2, 3) BESeg-1, 4) BE+TV and 5) BE+HPF for a representative patient (Male, 15 years) with osteosarcoma in right femur at three time-points – A: Baseline (t1); B: 1st Follow-up (t1); and C: 2nd Follow-up (t2).

For A, B and C: i) DWI (b=800 sec/mm^2) with ROIs for tumor (red outline) and healthy tissue (blue outline); ii) Data fitting in tumor and iii) Data fitting in healthy tissue by five IVIM analysis methods. In all the plots, along X-axis: b-values (0 – 800 sec/mm^2) and along Y-axis: relative signal intensity. Fitting curves in the range, b-value = 0 – 100 sec/mm^2 are enlarged in the inset.

Figure 2.6: i, iv, vii) DWI (b=800 sec/mm^2); ii, v, viii) DWI with ROIs for tumor (red) and healthy tissue (blue); iii, vi, ix) Apparent Diffusion coefficient (*ADC*), of a representative patient (Male, 15 years) with osteosarcoma in right femur (same tumor slice as in Figure 2.4) at 3 time-points t0, t1 and t2 respectively. IVIM parametric maps estimated by five IVIM analysis methodologies 1) BE, 2) BESeg-2, 3) BESeg-1, 4) BE+TV and 5) BE+HPF at time-points t0, t1 and t2 are depicted in A) Diffusion coefficient (*D*), B) Perfusion coefficient (*D**) and C) Perfusion fraction (*f*). [Figure 2.5 B, C continued in next page]

Figure 2.7: Bland-Altman plots showing inter-scan agreement of estimated parameters, Diffusion coefficient (a, b c), Perfusion coefficient (d, e, f) and Perfusion fraction (g, h, i) by IVIM analysis method BE+TV between time-points t0 and t1 (1st column); time-points t0 and t2 (2nd column); and time-points t1 and t2 (3rd column) in healthy tissue volume.

Figure 2.8: Bland-Altman plots showing inter-scan agreement of estimated parameters, Diffusion coefficient (a, b c), Perfusion coefficient (d, e, f) and Perfusion fraction (g, h, i) by IVIM analysis method BE+HPF between time-points t0 and t1 (1st column); time-points t0 and t2 (2nd column); and time-points t1 and t2 (3rd column) in healthy tissue volume.

Figure 3.1: a) DWI (b=800 sec/mm^2) axial slices of a representative patient (M, 15 years) with osteosarcoma in right distal femur; b) ROIs drawn for healthy (blue overlay) and tumor (red overlay) tissue; c) Original and fitted signals for BE+TV method in healthy and tumor tissue ROIs;

d) Apparent diffusion coefficient (*ADC*); e) Diffusion coefficient (*D*); f) Perfusion coefficient (*D**); g) Perfusion fraction (*f*).

Figure 3.2: a-q) Images of a representative patient from PR group (M, 16 years) with OS in left tibia and tumor-diameter was 6.6cm, 6.5cm and 4cm at time points t0, t1 (Δ1:2% decrement) and t2 (Δ2:33% decrement) respectively. a,b) T2W fat-saturated image showing anatomical structure at time-points t0 and t2 respectively; c,d,e) DWI (b=800 sec/mm^2); f,g,h) Apparent diffusion coefficient (*ADC*); i,j,k) Diffusion coefficient (*D*); l,m,n) Perfusion coefficient (*D**); o,p,q) Perfusion fraction (*f*) at time points t0, t1 and t2 respectively. Both *ADC & D* in tumor demonstrated an increase; *D** showed a decrease; *f* did not show much difference in tumor ROI after chemotherapy.

Figure 3.3: a-q) Images of a representative patient from SD group (F, 15 years) with OS in left tibia in whom tumor-diameter was 8.5cm, 10cm and 7.2cm at time points t0, t1 (Δ1:18% increment) and t2 (Δ2:15% decrement) respectively. a,b) T2W fat-saturated image showing anatomical structure at time-points t0 and t2 respectively; c,d,e) DWI (b=800 sec/mm^2); f,g,h) Apparent diffusion coefficient (*ADC*); i,j,k) Diffusion coefficient (*D*); l,m,n) Perfusion coefficient (*D**); o,p,q) Perfusion fraction (*f*) at time points t0, t1 and t2 respectively. Both *ADC&D* in tumor demonstrated an increase; whereas *D** showed an increment at t1, followed by a decrease at t2; *f* did not show much difference in tumor ROI after chemotherapy.

Figure 3.4: a-q) Images of a representative patient from PD group (F, 30 years) with OS in left tibia in whom tumor-diameter was 10.4cm, 12.6cm and 11.5cm at time points t0, t1 (Δ1:21% increment) and t2 (Δ2:11% increment) respectively. a,b) T2W fat-saturated image showing anatomical structure at time-points t0 and t2 respectively; c,d,e) DWI (b=800 sec/mm^2); f,g,h) Apparent diffusion coefficient (*ADC*); i,j,k) Diffusion coefficient (*D*); l,m,n) Perfusion coefficient (*D**); o,p,q) Perfusion fraction (*f*) at time points t0, t1 and t2 respectively. Both *ADC&D* in tumor demonstrated an increase; whereas *D** showed a decrement after at t1, followed by an increase at t2; *f* did not show much difference in tumor ROI after chemotherapy.

Figure 3.5: Images of a representative patient from Response group (M, 18 years), with OS in right distal femur and >90% histological necrosis after surgery. 1st, 2nd and 3rd columns are showing images at time points t0 (before Chemotherapy), t1 (after 1st cycle of chemotherapy) and t2 (after completion of chemotherapy) respectively and 4th column is representing histograms of parametric maps at three time-points t0 (green), t1 (orange) and t2 (blue) mentioned.

a, b) T2-weighted fat saturated image; c, d, e) DWI (b=800 sec/mm^2); f, g, h) Apparent diffusion coefficient (*ADC*); i) Histogram of *ADC*; j, k, l) Diffusion coefficient (*D*). m) Histogram of *D;* n, o, p) Perfusion coefficient (*D**). q) Histogram of *D*; r, s, t) Perfusion fraction (*f*). u) Histogram of *f;* v, w, x) D**.f;* y) Histogram of D**.f* .

Figure 3.6: Images of a representative patient from Non-Response group (M, 12 years), with OS in left tibia and <25% histological necrosis after surgery. 1st, 2nd and 3rd columns are showing images at time points t0 (before Chemotherapy), t1 (after 1st cycle of chemotherapy) and t2 (after completion of chemotherapy) respectively and 4th column is representing histograms of parametric maps at three time-points t0 (green), t1 (orange) and t2 (blue) mentioned.

a, b) T2-weighted fat saturated image; c, d, e) DWI (b=800 sec/mm^2); f, g, h) Apparent diffusion coefficient (*ADC*); i) Histogram of *ADC*; j, k, l) Diffusion coefficient (*D*). m) Histogram of *D;* n, o, p) Perfusion coefficient (*D**). q) Histogram of *D*; r, s, t) Perfusion fraction (*f*). u) Histogram of *f;* v, w, x) D**.f;* y) Histogram of D**.f* .

Figure 3.7: ROC curve analysis using mean and statistically significant (p<0.05) histogram parameters of *ADC, D, D*, f* and *D*.f* .

a) At time point t0 (before commence of chemotherapy), parameters in combination produced AUC=0.92, sensitivity=85%, Specificity=92% in predicting Non-responders to chemotherapy.
b) At time point t1, (after completion of 1st chemotherapy cycle), parameters in combination produced AUC=0.9, sensitivity=83%, specificity=92% in predicting Non-responders to chemotherapy.

Figure 4.1: A) Simple Linear Iterative Clustering Supervoxels and Ostu Multi-Thresholding (SLICs+MTh) based sub-segmentation of Osteosarcoma into different pathological parts like edema, viable tumor and necrosis using Diffusion Weighted Imaging (DWI) at diffusion weighting factor b=800sec/mm^2 (DWI_{800}), T2 weighted fat-saturate (T2W-fatsat) image and Apparent Diffusion Coefficient (*ADC*) map at baseline (pre-treatment) and 2nd follow-up (after completion of chemotherapy).

c) B) Flow-diagram of proposed SLICs+MTh based sub-segmentation method.

Figure 4.2: MRI images of one tumor slice from a representative patient (M, 16yrs) with Osteosarcoma in left tibia at A. Baseline (before commencement of chemotherapy) and B. 2nd follow-up (after completion of chemotherapy).

For A and B: a) T2 weighted fat-saturated images with demarked ROIs (Ground-Truth mask, red outline) for hyper-intense tumor and associated edema; b) SLICs+MTh based segmentation mask for tumor and associated edema (Mask1, red overlay) c) Diffusion weighted image at diffusion weighting factor b=800 sec/mm^2 (DWI_{800}) with demarked ROIs (Ground-Truth mask, blue outline) for hyper-intense tumor areas; d) SLICs+MTh based segmentation mask for tumor areas (Mask2, blue overlay); e) Apparent diffusion coefficient (*ADC*) with demarked ROIs (Ground-Truth mask, green outline) for possible hyper-intense necrosis areas; f) SLICs+MTh based segmentation mask for necrosis areas (Mask3, green overlay).

Figure 4.3: Box and whisker plots show the distribution of a) *ADC*-mean, b) *ADC*-entropy and c) hyper-parameter *ADC*-(mean×entropy) values in patient cohort.

Figure 4.4: a) Box and whisker plot showing amount of tumor necrosis computed by SLICs+MTh method at baseline and 2nd follow-up for patient groups below and above 50% histopathological necrosis and the amount of actual necrosis assessed by histopathological examination after surgical resection. There was no significant difference (p=0.26) between SLICs-based necrosis at follow-up and histopathological necrosis among all patients.

b) Bland-Altman plot showing agreement between tumor necrosis computed by SLICs+MTh method at 2nd follow-up and histopathological necrosis for all patients.

Figure 4.5: a) SLICs+MTh based segmented necrosis area in axial *ADC* map (blue outline) of a representative patient (F, 27years) with osteosarcoma in left femur from Responder group; b) Demarcated necrosis area (red outline) in corresponding post-contrast T1W image from the same patient. HPE slide images at c) and d) depict large area of necrosis of different components of osteosarcoma (solid arrow) and e) shows area of viable tumor with hyalinization (hollow arrow).

f) SLICs+MTh based necrosis area in axial *ADC* map (blue outline) of a representative patient (F, 16years) with osteosarcoma in right distal femur from No-responder group; g) Demarcated necrosis area (red outline) in corresponding post-contrast T1W slice from the same patient. HPE slide images at h), i) and j) depict large viable areas of osteosarcoma (hollow arrow) and area of necrosis (solid arrow).

Figure 4.6. A, B: SLICs+MTh based segmentation of DWI, T2W-fatsat and *ADC* map and mask generation for tumor, edema and necrosis in a tumor slice of a representative patient (F, 12 years) from Responder group with osteosarcoma in right femur at A. Baseline and B. 2nd follow-up. For A and B: a, d, g) DWI (b=800 sec/mm^2), T2W-fatsat, *ADC* map respectively; b, e, h) Supervoxels generation on DWI, T2W-fatsat, and *ADC* map respectively; c, f, i) Segmented Mask(tumor), Mask(tumor+edema) and Mask(necrosis) from b, e and h respectively; j) Final segmentation.

Figure 4.7. A, B: SLICs+MTh based segmentation of DWI, T2W-fatsat and *ADC* map and mask generation for tumor, edema and necrosis in a tumor slice of a representative patient (M, 16 years) from Non-responder group with osteosarcoma in left tibia at A. Baseline and B. 2nd follow-up. For A and B: a, d, g) DWI (b=800 sec/mm^2), T2W-fatsat, *ADC* map respectively; b, e, h) Supervoxels generation on DWI, T2W-fatsat, and *ADC* map respectively; c, f, i) Segmented Mask(tumor), Mask(tumor+edema) and Mask(necrosis) from b, e and h respectively; j) Final segmentation.

Figure 5.1: Eight nearest-neighbor pixels are used to describe pixel connectivity for calculating GLCM for an image. Cells 1 and 5 show the horizontal (P_H), 4 and 8 the right-diagonal (P_{RD}), 3 and 7 the vertical (P_V) and 2 and 6 the left-diagonal (P_{LD}) nearest-neighbors of the pixel P at center.

Figure 5.2: Example of an 4x4 image with four unique gray-levels and calculation of GLCM in horizontal direction (P_H) at a co-occurrence distance of 1.

Figure 5.3: Example demonstrates the formation of a NGTDM (201). Left, 5x5 image with five unique gray-levels. Right, the resulting NGTDM.

Figure 5.4: Example demonstrates the formation of a RLM. Left, 4x4 image with four unique gray-levels. Right, the resulting RLM in the direction 0^0.

Figure 5.5: T1W, T2W non-fat-saturated and fat-saturated, DWI_{800} images and *ADC, D, D** and *f* parametric maps from a representative patient (M, 16years) with osteosarcoma in left tibia with ROIs drawn for tumor (red) and healthy (blue) tissue.

Figure 5.6: A. Images of a representative patient (Male, 19 years) from Non-survivor group with osteosarcoma in right tibia at baseline.
B. Images of a representative patient (Male, 18 years) from Survivor group with osteosarcoma in left femur at baseline.
For A and B: a) T2-weighted; b) DWI (b=800 sec/mm^2); c) Apparent diffusion coefficient (*ADC*); d) Diffusion coefficient (*D*); e) Perfusion coefficient (*D**) and f) Perfusion fraction (*f*).

Figure 5.7: Distribution of statistically significant (p<0.05) textural features a) Histogram: mean, b) GLCM: sum-average, c) GLCM: variance, d) GLCM: autocorrelation, e) NGTDM: coarseness, f) NGTDM: busyness, g) NGTDM: strength and h) RLM: HGRE in *D** map producing AUC≥0.75 in classifying among Survivor and Non-survivor groups at baseline.

Figure 5.8: A. a-u) Images of a representative patient from Res group (Male, 18 years, >90% necrosis in histopathological assessment) with osteosarcoma in right distal femur.
B. a-u) Images of a representative patient from NRes group (Male, 18 years, <30% necrosis in histopathological assessment) with osteosarcoma in left femur (same patient in Figure 5.2.B).
For A and B: a, b) T1W; c, d) T2W nonfat-saturated; e, f) T2W fat-saturated images at baseline (t0) and 2nd follow-up (t2) respectively; g,h,i) DWI (b=800 sec/mm^2); j, k, l) Apparent diffusion coefficient (*ADC*); m, n, o) Diffusion coefficient (*D*); p, q, r) Perfusion coefficient (*D**); s, t, u) Perfusion fraction (*f*) at baseline (t0), 1st follow-up (t1) and 2nd follow-up (t2) respectively.

Figure 5.9: Distributions of statistically significant (p<0.05) textural features among response groups effective in evaluating chemotherapy response in osteosarcoma before or early in the course of treatment. Individually these features produced sensitivity & specificity of 70-90% for both and AUC=0.70-0.83 in predicting Responder and Non-responder groups:
a) f_{13} (NGTDM: complexity) in T2W-nonfatsat; b) f_2, (GLCM: contrast), c) f_5 (GLCM: correlation) and d) f_{22} (RLM: SRLGE) in D* map; e) f_2, (GLCM: contrast), f) f_5 (GLCM: correlation), g) f_{11}, (NGTDM: contrast) and h) f_{13}, (NGTDM: complexity) in *f* map.

Appendix I Figure 1: Estimated parameter maps for Diffusion Coefficient (*D*), Perfusion Coefficient (*D**) and Perfusion Fraction (*f*) by BE, BEseg-1, BSseg-2, BE+TV and BE+HPF methods at SNR=25.

Appendix I Figure 2: Relative Bias (1st row), relative SD of error (2nd row), and relative RMS error (3rd row) of the IVIM parameter estimation methods BE, BEseg-1, BEseg-2, BE+TV, BE+HPF at different SNR levels of 100, 50, 25, 12.5.

Appendix III Figure 1: Illustrative example of a) T2 weighted fat-saturated image; b) Diffusion weighted image with b=800sec/mm^2; c) Manual annotation and measurement of maximum Tumor-diameter; d) Manually drawn region of interest for tumor, from a representative patient (M, 15y) with osteosarcoma in left tibia.

Appendix III Figure 2: Illustrative example of Tumor segmentation using SLIC-Supervoxels and Fuzzy C-mean Clustering algorithms and Tumor-diameter evaluation using Connected Component analysis, from a representative patient (M, 15y) with osteosarcoma in left tibia.

Appendix III Figure 3: a) T2W fat-saturated image and b) DWI (b=800 sec/mm^2) of a representative patient (M, 15 years) with osteosarcoma in right tibia; c) Ground-truth for tumor ROI (red outline) demarcated by radiologist; Gray-level Co-occurrence matrix (GLCM) feature maps: d) Contrast; e) Correlation; f) Energy; g) Homogeneity and Gray-Level Run-Length Matrix (GLRLM) feature maps: h) Short Run Emphasis (SRE); i) Long Run Emphasis (LRE); j) Gray-Level Nonuniformity (GLN); k) Run-Length Nonuniformity (RLN); l) Run Percentage (RP); m) Low Gray-Level Run Emphasis (LGRE); n) High Gray-Level Run Emphasis (HGRE); o) Short Run Low Gray-Level Emphasis (SRLGE); p) Short Run High Gray-Level Emphasis (SRHGE); q) Long Run Low Gray-Level Emphasis (LRLGE) and r) Long Run High Gray-Level Emphasis (LRHGE) for input b) DWI (b-value=800 sec/mm^2).

Appendix III Figure 4.A, B, C & D: Illustrative examples of segmentation results of nine implemented methods.

A) A representative patient (Male, 15years) with OS in right tibia; B) A representative patient (Male, 15years) with OS in right femur; C) A representative patient (Male, 17years) with OS in right humerus; D) A representative patient (Female, 16years) with OS in iliac bone.

A, B, C & D. a) T2W fat-saturated image; b) DWI (b=800 sec/mm^2); c) Ground-truth for tumor ROI (red outline) demarcated by radiologist; Segmentation results (blue outline) by d) Otsu-thresholding; e) Otsu-threshold-based region growing; f) Active contours; g) SLIC-Superpixels; h) Fuzzy c-means clustering; i) Graph cut; j) Logistic regression; k) Linear support-vector-machines; l) Deep feed-forward neural-network. Automated SLIC-Superpixels, Fuzzy c-means

clustering and semi-automated Otsu-threshold-based region growing, Active contours methods produced qualitatively more accurate segmentation than other methods.

Appendix III Figure 5.A, B & C: Illustrative examples of segmentation results of SLICs and FCM methods at baseline and 2nd follow-up for **A)** A representative patient (Male, 15years) with OS in right humerus having ground-truth RECIST1.1 score: Partial responder (PR); **B)** A representative patient (Male, 15years) with OS in left femur having ground-truth RECIST1.1 score: Stable disease (SD); **C)** A representative patient (Male, 18years) with OS in right shoulder having Ground-truth RECIST1.1 score: Progressive disease (PD).

For A, B &C. a, e) axial DWI (b=800 sec/mm^2) with maximum cross-sectional area of tumor burden; b, f) ground-truth for tumor ROI (red outline) demarcated by radiologist; c, g) SLIC-segmentation (green outline); d, h) FCM segmentation (yellow outline) at baseline and 2nd follow-up respectively.

Appendix III Figure 6: Comparison of ground-truth Tumor-Diameters and ground-truth Tumor-Volume with automatically assessed measurements applying A. SLICs and B. FCM methods in patient cohort.

For A, B a, b) Bland Altman plot showing agreement between ground-truth Tumor-diameter by observer-1 and automatically assessed tumor-diameter at baseline and 2nd follow-up respectively;

c, d) Bland Altman plot showing agreement between ground-truth Tumor-diameter by observer-2 and automatically assessed tumor-diameter at baseline and 2nd follow-up respectively;

e, f) Bland Altman plot showing agreement between ground-truth Tumor-volume and automatically assessed tumor-volume at baseline and 2nd follow-up respectively.

LIST OF TABLES

Table 2.10: Average *ADC, D*, *D** and *f* values in tumor volume evaluated by five IVIM analysis methods at three time-points.

Table 2.11: Average *ADC, D*, *D** and *f* values in healthy tissue volume evaluated by five IVIM analysis methods at three time-points.

Table 2.12: Mean±Std-Dev of *D*, *D** and *f* parameter values in healthy tissue volume evaluated by BE+TV and BE+HPF IVIM analysis methods at time-points t0, t1 and t2 and their inter-scan comparison of mean parameter values, within-subject coefficient of variation (wCV) and Bland-Altman agreement across time-points.

Table 3.1: Mean ± Std-Dev Apparent Diffusion Coefficient (*ADC*), Diffusion coefficient (*D*), Perfusion coefficient (*D**), Perfusion fraction (*f*) and *D*.f* in healthy and tumor tissue ROI volume in patient cohort at time-point t0 (before commencement of chemotherapy). Estimated parameters in healthy and tumor tissue were significantly different ($p<0.05$).

Table 3.2: Average Tumor-diameter (cm) and Tumor-volume (cc) measured among different response groups viz. Partial-response (PD), Stable-disease (SD) and Progressive-disease (PD) at three time-points t0, t1 and t2 in the course of neoadjuvant chemotherapy.

Table 3.3: Histogram parameters of Apparent diffusion coefficient (*ADC),* Diffusion-coefficient *(D),* Perfusion-coefficient *(D*) &* Perfusion-fraction *(f)* observed in different response groups: Partial-response (PR), Stable-disease (SD) and Progressive-disease (PD) at time points t0, t1 and t2 in the course of chemotherapy.

Table 3.4: Statistically significant ($p<0.05$) parameters according to ANOVA test at three time-points t0, t1 & t2 and their respective Tukey post hoc test among response groups. Statistically significant ($p<0.05$) *p*-values are in **'bold'.**

Table 3.5: Pearson-correlation-coefficient (*r*) of relative percentage changes in Tumor-diameter and Tumor-volume across different time points (Δ1 & Δ2) with relative percentage changes of histogram parameters of diffusion and perfusion parameters *ADC*, *D*, *D** and *f* among all patients.

Table 3.6: Histogram parameters of Apparent diffusion coefficient (*ADC),* Diffusion-coefficient *(D),* Perfusion-coefficient *(D*) &* Perfusion-fraction *(f)* observed in different response groups: Responder (Res) and Non-responder (NRes) at time points t0 (before chemotherapy), t1 (after 1st

cycle of chemotherapy) and t2 (after completion of chemotherapy) and their relative percentage changes between time points t0 & t1 (Δ1) and t0 & t2 (Δ2). Statistically significant (p<0.05) values are in **'bold'**.

Table 3.7: Statistically significant (p<0.05) histogram parameters of diffusion and perfusion parameters among Responder and Non-Responder groups at time-points t0 (before chemotherapy) and t1 (after 1st cycle of chemotherapy) and their ROC curve analysis.

Table 4.1: Mean±standard deviation of Jaccard-index (JI), Dice-coefficient (DC), Precision (P) and recall (R) for calculated Mask1 (tumor+edema), Mask2 (tumor), Mask (edema) and Mask (necrosis) in all patients for baseline and 2nd follow-up data after 3 cycles of Neoadjuvant chemotherapy (NACT).

Table 5.1: First-order statistical texture/ Histogram analysis features.

Table 5.2: Texture features calculated by statistical texture analysis method GLCM.

Table 5.3: Texture features calculated by statistical texture analysis method NGTDM.

Table 5.4: Texture features calculated by statistical texture analysis method RLM.

Table 5.5: List of different texture features calculated by statistical texture analysis methods: Gray Level co-occurrence matrix (GLCM) (feature: f_1-f_9), Neighborhood gray-tone difference matrix (NGTDM) (feature: f_{10}-f_{14}) and Run length matrix (RLM) (feature: f_{15}-f_{25}).

Table 5.6: Statistically significant (p<0.01) texture features for classifying healthy and tumor tissue using DWI_{800}, T1W, T2W-nonfatsat and T2w-fatsat images. Texture features individually showing sensitivity and specificity of ≥90% for both with AUC ≥ 0.9 for classification among healthy and tumor tissue are in **'bold'**. In first bracket combined ROC analysis results for significant features are represented showing Sn: Sensitivity, Sp: Specificity, AUC: Area under curve.

Table 5.7: Statistically significant (p<0.05) textural features for classification between Survivor and Non-survivor groups. Textural features individually showing AUC ≥ 0.75 for classification between two groups are in **'bold'**. In the first bracket combined ROC curve analysis results for significant features for each texture analysis method are presented showing Sn: Sensitivity, Sp: Specificity, AUC (CI): Area under curve (95% confidence interval).

Table 5.8: Selected textural features for identifying Responders and Non-responders to chemotherapy in all parametric maps and MR images. Textural features individually showing AUC≥0.7 for classification between the two groups are in '**bold'**. Combined ROC analysis results for selected features for each texture analysis (TA) method are presented in the first bracket. Combined ROC analyses for selected features for all TA methods are presented in vertical columns. Sn: Sensitivity, Sp: Specificity, AUC (CI): Area under curve (95% confidence interval).

Table 6.1: Textural feature(s) estimates for generalized linear regression model to predict tumor aggressiveness at baseline (t0) and responsiveness to chemotherapy in the course of chemotherapy (t0, t1 and t2).

Appendix II Table 1: Mean±Std-Dev values of textural features in tumor and healthy tissue volume evaluated in DWI (b=800 sec/mm^2) at baseline. Statistically significant (p<0.01) features are in **'bold'**.

Appendix II Table 2: Mean±Std-Dev values of textural features in tumor and healthy tissue volume evaluated in T1-weighted MRI at baseline. Statistically significant (p<0.01) features are in **'bold'**.

Appendix II Table 3: Mean±Std-Dev values of textural features in tumor and healthy tissue volume evaluated in T2-weighted non-fat-saturated MRI at baseline. Statistically significant (p<0.01) features are in **'bold'**.

Appendix II Table 4: Mean±Std-Dev values of textural features in tumor and healthy tissue volume evaluated in T2-weighted fat-saturated MRI at baseline. Statistically significant (p<0.01) features are in **'bold'**.

Appendix II Table 5: Area under the curve (AUC) analysis of statistically significant (p<0.01) textural features for predicting tumor aggressiveness leading to mortality at baseline (t0). CI: 95% confidence interval. Biomarkers with AUC≥0.75 are in **'bold'**.

Appendix II Table 6: Area under the curve (AUC) analysis of selected textural features for predicting Non-responders to chemotherapy at baseline (t0). CI: 95% confidence interval. Biomarkers with AUC≥0.7 are in **'bold'**.

Appendix III Table 1: Summary of nine segmentation algorithms implemented in this study.

Appendix III Table 2: Mean ± Std-Dev Dice-Coefficient (*DC*), Jacquard-Index (*JI*), Precision (*P*), Recall (*R*), execution time, Apparent diffusion coefficient (*ADC)* values and corresponding Pearson-correlation-coefficient (*r*) with Ground-truth *ADC* across patients with osteosarcoma for nine implemented methods (Tumor *ADC* for ground-truth mask: 1.3±0.33 x10^3 mm^2/sec).

Appendix III Table 3: Tumor-diameter (cm), Tumor-volume (cc) and tumor *ADC* in patient cohort. Segmentation accuracy metrics Dice-Coefficient (*DC*), Jacquard-Index (*JI*), Precision (*P*), Recall (*R*) and Pearson-correlation-coefficient (*r*) of measured parameters with ground-truth measurements across patients and overall execution time (second)/patient for SLICs and FCM methods. Values are showing Mean±Std-Dev in patient cohort.
Ground-truth Tumor-diameter at baseline: 9.72±2.88cm; 10.27±3.17 and at 2nd follow-up: 8.51±3.03cm; 8.85±3.38cm for observer-1 and observer-2 respectively;
Ground-truth Tumor-volume at baseline: 514.26±504.57cc and at 2nd follow-up: 435.97±413.29cc; *ADC* for ground-truth Tumor-volume at baseline: 1.3±0.33x10^3 mm^2/sec and at 2nd follow-up: 1.72±0.27x10^3 mm^2/sec.

Appendix III Table 4: Total number of patients in four response group (CR, PR, SD & PD) according RECIST1.1 score and Volumetric response score determined by manual method and automated methods using SLICs and FCM.

GLOSSARY

Abbreviation	Full form / Meaning
1D	One-dimensional
3D	Three-dimensional
AC	Active contour
ACC	average correlation coefficients
ADC	Apparent diffusion coefficient
AIIMS	All India Institute of Medical Sciences
AUC	Area under curve
bCV	Between-subject coefficient of variation
BE	Bi-exponential
BEseg-2	Segmented BE method with 2-parameter fitting
BEseg-1	Segmented BE method with 1-parameter fitting
BE+TV	BE model with Total Variation Penalty function
BE+HPF	BE model with Huber Penalty function
BF	Blood flow
BV	Blood volume
b-value	Diffusion weighting factor
CAD	Computer aided diagnostic
CBV	Cerebral blood volume
CR	Complete-response
CT	Computed tomography
D	Diffusion coefficient
D^*	Perfusion coefficient
DC	Dice-coefficient
DCE	Dynamic contrast enhanced
D_{rel}	Relative diffusion coefficient
D^*_{rel}	Relative perfusion coefficient
DNN	Deep feed-forward neural-network
DSC	Dynamic susceptibility contrast-enhanced
DW	Diffusion weighted

DWI	Diffusion weighted imaging
DWI_{800}	DWI with b-value=800sec/mm^2
Δ1	Relative percentage changes between t0–t1
Δ2	Relative percentage changes between t0–t2
ESFT	Ewing's sarcoma
f	Perfusion fraction
FCM	Fuzzy c-means clustering
FDG	Fluorodeoxyglucose
f_{rel}	Relative Perfusion fraction
GC	Energy-based graph cut
GLCM	Gray-level co-occurrence matrix
GLN	Gray-Level Nonuniformity
GLRM	Generalized linear regression model
GMs	Gradient magnitudes
GTSDM	Gray-tone spatial dependence matrices
HCC	Hepatocellular carcinoma
HGRE	High Gray-Level Run Emphasis
HNSCC	Head and neck squamous cell carcinoma
HPE	Histopathological evaluation
HPF	Huber penalty function
IVIM	Intravoxel Incoherent Motion
JI	Jacquard-index
KS test	Nonparametric two-sample Kolmogorov-Smirnov test
LARC	locally advanced rectal cancer
LGRE	Low Gray-Level Run Emphasis
LM	Levenberg-marquardt
LR	Logistic regression
LRE	Long Run Emphasis
LRHGE	Long Run High Gray-Level Emphasis
LRLGE	Long Run Low Gray-Level Emphasis
L-SVM	Linear support-vector-machines
Mth	Otsu multi-thresholding
MR	Magnetic resonance
MRI	Magnetic resonance imaging

ME	Mono-exponential
MER	Misclassification error rate
MTT	Mean transit time
MWW	Mann-Whitney-Wilcoxon
NACT	Neoadjuvant chemotherapy
NHTDM	Neighborhood gray-tone difference matrix
NLLS	Non-linear least-squares
NRes	Non-responder
OS	Osteosarcoma
OT	Otsu-thresholding
OT-RG	Otsu-threshold-based region growing
P	Precision
P_H	Horizontal
P_{LD}	Left-diagonal
P_{RD}	Right-diagonal
P_V	Vertical
PD	Progressive-disease
PET	Positron emission tomography
PGSE	Pulsed Gradient Spin Echo
POE	classification error probability
PR	Partial response
PWI	Perfusion weighted imaging
r	Pearson-correlation-coefficient
R	Recall
R^2	Coefficient-of-determination
RB	Relative bias
RF	Radio frequency
ROI	Region of interest
RECIST	Response evaluation criteria in solid tumors
Res	Responder
RLM	Run length matrix
RLN	Run Length Nonuniformity
ROC	Receiver-operating-characteristic-curve
RP	Run Percentage

RPC	Relative percentage change
RRMSE	Relative root mean-square error
S_b	Diffusion weighted signal/image at diffusion weighting factor value b sec/mm^2
SD	Stable disease
SLICs	Simple linear iterative clustering supervoxels
SLICs+MTh	Simple linear iterative clustering supervoxel and Otsu multi-thresholding
Sn	Sensitivity
SNR	Signal to Noise Ratio
$SNRs_0$	SNR in diffusion weighted image S_0
Sp	Specificity
SRE	Short Run Emphasis
SRHGE	Short Run High Gray-Level Emphasis
SRLGE	Short Run Low Gray-Level Emphasis
Std-Dev	Standard deviation
SUV	Standardized uptake value
t0	Baseline (before chemotherapy)
t1	1st follow-up (after 1st cycle of chemotherapy)
t2	2nd follow-up (after 3rd cycle of chemotherapy)
T1W	T1 Weighted
T2W	T2 Weighted
T2W-fatsat	T2-weighted fat-saturated
T2W-nonfatsat	T2-weighted non-fat-saturated
TA	Texture analysis
TE	Time of echo
Th	Threshold
TR	Repetition time
TV	Total variation
VOI	Volume of interest
wCV	Within-subject coefficient of variation