

Histological aspects of post-TACE hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer in men and the seventh in women and is the third most common cause of death from cancer worldwide [<http://globocan.iarc.fr>]. The overall incidence of HCC remains high in developing countries and is steadily rising in most industrialized countries [Shariff MI *et al.*, 2009]. A variety of therapeutic modalities is available for treating hepatocellular carcinoma, but orthotopic liver transplantation (OLT) represents a curative option. Due to the shortage of donor organs and the increasing need for liver transplantation in the last decade, local ablation therapy (LAT) has been increasingly used in many centers as a bridge to transplant [Majno PE *et al.*, 1997; Decaens T *et al.*, 2005; Herber S *et al.*, 2005; Bharat A *et al.*, 2006; Obed A *et al.*, 2007; Otto G *et al.*, 2007]. We retrieved from the archive in the Histopathology Laboratory, Institute of Liver Studies, King's College Hospital, London, UK, 28 cases of HCC, which underwent treatment with TACE (Doxorubicin 40 mg/m²) as a bridge to transplantation, between 2008 and 2010. We also analyzed 14 additional post-TACE tumors, classified according to the architectural patterns published by Morisco F *et al.* (2008), for quantification of necrosis. Extensive tumor necrosis was observed in 12 (42.85%) of the patients. Viable hepatocellular carcinoma showed a wide range of differentiation, from well to poorly differentiated. The phenotype of the tumors was mostly hepatocellular, but 14% showed a mixed phenotype, including glandular/pseudoglandular formation and cholangiocellular components. The percentage of necrosis ranged between 0% and 100%, with an average of 50.6%. There was no statistical correlation between the total size of the nodules and the surface of necrosis in our series ($p=0.125$). In conclusion, the systematic pathological assessment of post-TACE resected HCC can help in investigating the biology of treated tumors but needs to incorporate sampling protocols, digital image analysis, phenotypic classification by immunohistochemistry and enzymatic function.

Keywords: hepatocellular carcinoma, TACE, laser microdissection, necrosis.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer in men and the seventh in women and is the third most common cause of death from cancer worldwide [1]. The overall incidence of HCC remains high in developing countries and is steadily rising in most industrialized countries [2]. It is long known that the major clinical risk factor for HCC is liver cirrhosis, largely independent of its etiology [3, 4].

A variety of therapeutic modalities is available for treating hepatocellular carcinoma (HCC), but orthotopic liver transplantation (OLT) represents the only curative option. Through OLT, both the tumor and the underlying cirrhosis can be cured [4–6]. Due to the shortage of donor organs and the increasing need for liver transplantation in the last decade, local ablation therapy (LAT) has been increasingly used in many centers as a bridge to transplant [7–14]. Of all the different LAT modalities, trans-arterial chemoembolization (TACE) is the treatment of choice in many centers because it exploits the predominant arterial supply to HCC and combines ischemic injury with chemotherapeutic toxicity [15]. Besides downstaging patients, the most important goal of TACE in transplant candidates is to

keep them in a steady state and to avoid dropout from the transplant list [9–13].

TACE efficacy is usually assessed by imaging and monitoring tumor markers [15, 16]. Complete response is achieved in fewer than 2% of patients, the rate of objective response ranging from 16% to 60% [17]. The effect of TACE or other modalities of local ablation therapy on treated tumors can also be evaluated at tissue level when the whole liver is subsequently removed at transplantation [18].

Materials and Methods

We retrieved from the archive in the Histopathology Laboratory, institute of Liver Studies, King's College Hospital, London, UK, 28 cases of HCC which underwent treatment with TACE (Doxorubicin 40 mg/m²) followed by transplantation, between 2008 and 2010.

The characteristics of the patients (*i.e.*, age, sex, and underlying chronic liver diseases) are summarized in Table 1.

Before the TACE treatment, all nodules were radiologically diagnosed to be HCC according to the *European Association for the Study of the Liver* criteria [17] for concordant imaging of nodular arterialized lesions with portal venous washout.

Table 1 – Age, sex, and underlying chronic liver diseases

Age [years]	58 (47–67)
Sex, male/female [n/n]	21/7
Etiology [n (%)]	
<i>Hepatitis B</i>	3 (10.7%)
<i>Hepatitis C</i>	13 (46.4%)
<i>Alcoholic</i>	5 (17.8%)
<i>Nonalcoholic steatohepatitis</i>	2 (7.1%)
<i>Budd–Chiari Syndrome</i>	1 (3.6%)
<i>Autoimmune hepatitis</i>	1 (3.6%)
<i>Autoimmune hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome</i>	1 (3.6%)
<i>Alpha-1 Antitripsin deficiency</i>	1 (3.6%)
<i>Cryptogenic</i>	1 (3.6%)

Livers removed during transplantation were received fresh, and they were sliced into parallel sections at approximately 1 cm intervals. Tumors were identified and samples were snap-frozen in *iso*-pentane and stored at -80°C . They were measured in three dimensions, the maximum diameters being recorded. The livers were reexamined after formalin fixation, and the tumors were sampled more extensively. Formalin-fixed tissue was embedded in paraffin, and the sections were stained with Hematoxylin and Eosin (HE). All post-TACE HCCs examined in this study had microscopic foci of viable hepatocellular carcinomas, as well as non-viable lesional tissue with features of coagulative necrosis, fibrous scarring, inflammation and granulation tissue consistent with the effects of therapy. The cases without viable tumors were not included. The viable component was assessed in terms of tumor differentiation and presence of features suggesting combined hepatocholangiocellular differentiation, such as the formation of ductules, glandular structures or other combined growth patterns [18]. The background liver, in sections away from the tumors, was also examined.

We also analyzed 14 additional post TACE tumors for quantification of necrosis. The explanted livers, from which these tumors were taken, were sliced into parallel 10 mm thick sections on a transverse plane in order to identify any focal lesion. Once a tumor was identified, it was cut into multiple parallel slices of 10 mm thickness using metal guides. Each slice was further bisected into two parallel thin slices of approximately 5 mm thickness, photographed using a Nikon Coolpix digital camera, attached to a specimen photography stand. All photographs were taken at the same magnification and distance from the specimen for all slices. A ruler was included in the photography field for reference measurements. Only the caudal side of each slice was photographed. After a photograph was taken the specimen was moved to the dissection bench and another photograph was taken using a Procam 1 camera connected to Pro-Path software (Ronse, Belgium) so that the position of the samples taken for histological analysis could be marked digitally. The samples were taken from various areas of the tumor so that areas of necrosis and areas of viable tissue could be identified

and matched to macroscopic digital images. All digitally acquired photographs were saved in JPEG format for subsequent image analysis. Histology sections with samples of each tumor were reviewed using an Olympus BX51 microscope. Liver parenchyma, in which hepatocytes maintain a liver cell plate structure with staining nuclei, was considered viable. Areas of non-viable tissue were identified as areas of confluent coagulative necrosis of liver parenchyma, fibrous scarring and/or granulation tissue. Each histological section was compared with the macroscopic picture and areas of non-viable tumor identified microscopically were identified in the corresponding specimen digital photograph. Judging from the macroscopic appearance of the non-viable areas proven microscopically, the areas of viable and non-viable tumor were identified in the rest of the digital photographs from the specimen slices.

The surface areas of viable and non-viable tumor were measured in each digital photograph using ImageJ 1.37v, Wayne Rasband, National Institute of Health, USA. The perimeter of each area was marked. The surface area was then calculated by the ImageJ using the “measure” function in the Analyze drop-down menu. Each measurement was transferred into an Excel table. After all areas of viable and non-viable tumor were measured, the total cumulative tumor surface for each tumor was calculated, and the percentage of non-viable parenchyma thus obtained.

Results

Seven (25%) of the 28 patients were females and 21 (75%) males. The age of the patients at the time of liver surgery ranged from 47 to 67 (mean 58 years).

All of the examined livers had tumors under 5 cm. Fourteen (50%) had a single tumor, seven (25%) had two tumors, two (7.15%) had three tumors, and five (17.85%), four or more tumors. On the examination of the background liver, 26 (92.85%) were cirrhotic and only two (7.15%) cases had bridging fibrosis, short of cirrhotic transformation.

Regarding the etiology of the tumors, 13 (46.4%) of them aroused in the context of HCV infection, five (17.8%) were alcohol induced and three (10.7%) in HBV infection. We also found one HCC (3.6%) within the liver of a patient with Budd–Chiari Syndrome, two (7.15%) in the context of non-alcoholic steatohepatitis (NASH), one (3.6%) in the context of alpha-1 Antitripsin deficiency and two (7.15%) following autoimmune conditions (autoimmune hepatitis and autoimmune hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome). One case (3.6%) had an unknown etiology.

The majority of the livers (15) had the tumors in the right lobe (53.6%), 10 of them in both the right and left lobes (35.7%), and just three only in the left lobe (10.7%).

From the total of 64 tumors examined, 50 (78.1%) had viable tumors and 14 (21.9%) were wholly necrotic. We, therefore, excluded seven (25%) of the 28 patients from further studies. Extensive tumor necrosis was observed in 12 (42.85%) of the patients.

Viable hepatocellular carcinoma showed a wide range of differentiation, from well to poorly differentiated. Twenty-three of the nodules (46%) identified were moderately differentiated hepatocellular carcinoma, eight (16%) of them were well differentiated and three (6%) poorly differentiated. There were also tumors with biphasic differentiation, 13 (26%) of them showing moderately differentiated and one (2%) well differentiated HCC, with areas of poor differentiation, and also two (4%) moderately differentiated with small well differentiated areas. Macroscopic or microscopic vascular invasion was present in 12 (57.1%).

The phenotype of the tumors was mostly hepatocellular, but seven (14%) of them showed a mixed phenotype, including glandular/pseudoglandular formation and cholangiocellular components. The etiology of this mixed phenotype tumors was HCV infection in four of them, the others being one alcohol-related, one Budd–Chiari Syndrome and one NASH.

Eleven of the 28 patients included in the study had elevated pre-TACE alpha-fetoprotein serum levels (39.3%). They underwent between one and four cycles of TACE, with an average time between the treatment and the transplant procedure of 7.1 months (ranging from one to 86 months).

At the present date, 26 (92.85%) of the patients are still alive, the overall mean survival being 35.7 months (range 19–77 months). We could not establish any correlation between the percentage of viable tumor and time interval between TACE and transplant, because the number of TACE cycles varied from patient to patient and also due to an insufficient number of cases for a valid statistical result.

The 14 tumors analyzed for the quantification of necrosis were classified according to the architectural patterns published by Morisco F *et al.* [16]:

- Pattern 1: Multiple nodules separated by fibrous septa;
- Pattern 2: Single nodule;
- Pattern 3: Single nodule with smaller adjacent satellite nodules (3a – viable satellite nodules, 3b – some necrotic satellite nodules) (Figure 1).

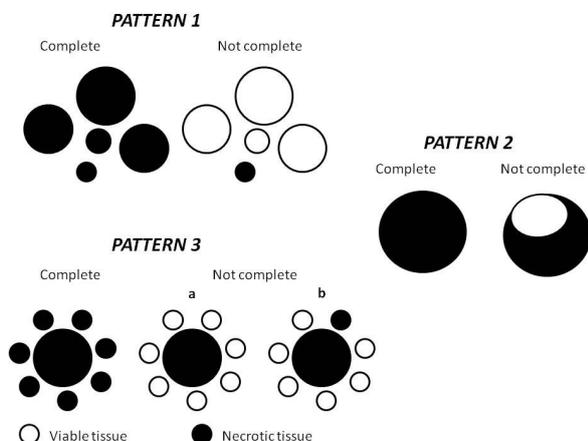


Figure 1 – Patterns of necrosis after local ablation therapy.

According to this classification, six (42.85%) were pattern 1, five (35.7%) pattern 2, one (7.14%) pattern 3a

and 2 (14.3%) pattern 3b tumors. The maximum diameter of the tumors ranged between 0.9 and 9 cm, with the highest calculated area of 125.4 cm² and the lowest one of 1.3 cm² (Figure 2).

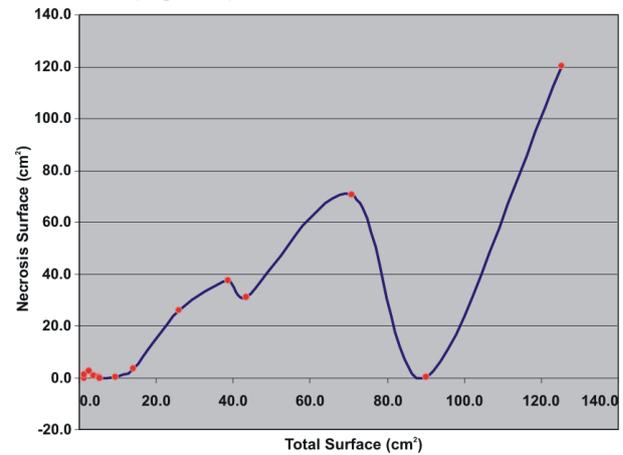


Figure 2 – The total surface of the tumors in relation with the tumoral necrotic surface.

The data following the measurements of the total surface, area of necrosis and necrotic percentage of the tumor areas are summarized in Table 2.

Table 2 – Percentage of necrosis and total, viable and necrotic surface of the tumors in relation with the pattern of necrosis

Tumor pattern	Total surface [cm ²]	Viable surface [cm ²]	Necrosis surface [cm ²]	Necrosis [%]
1	9.3	9	0.3	3.3
1	5.1	4.9	0.2	3.7
1	3.7	3.1	0.7	18.3
1	14	10.4	3.2	23.2
1	2.4	0.1	2.4	97.5
1	70.6	0	70.6	100
2	5.3	5.3	0	0
2	1.3	1.3	0	0
2	90.3	90	0.2	0.3
2	1.4	0	1.3	97.7
2	25.9	0	25.9	100
3a	125.4	5.1	120.3	95.9
3a	43.5	12.6	30.8	71
3b	38.7	1.1	37.6	97.2

The percentage of necrosis ranged between 0% and 100%, with an average of 50.6%. Two of the tumors underwent total necrosis (14.29%) and two of them showed no sign of necrosis. There was no statistical correlation between the total size of the nodule and the surface of necrosis in our series ($p=0.125$) (Figure 3).

Every third tumor within pattern 1 of the necrosis belonged to a different category of the ones mentioned above. Pattern 2 and 3 of the necrotic tumors were a part of category I/III and category III respectively.

One of the patients included in the study is not alive to present date due to a second malignancy (gastric cancer). Tumor recurrence after OLT and distant metastasis occurred in only two instances in which the tumor presented with pattern 3 necrosis and more than 90% unviable tissue.

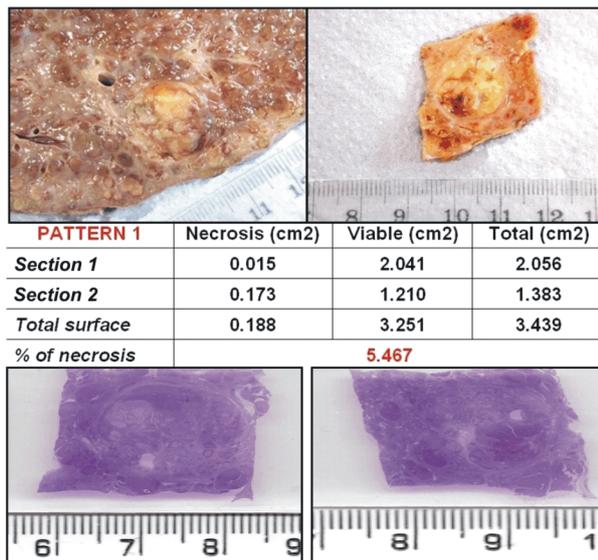


Figure 3 – Two of the sections used for the calculation of the percentage of necrosis.

Discussion

Assessment of tumor response after locoregional therapies is important in determining treatment success and in guiding future therapy [19].

According to practice guidelines [17] complete response to TACE is achieved in fewer than 2% of patients; the rate of objective response ranges from 16% to 60%; and extensive tumor necrosis occurs in more than 50% of patients [17].

In our study, 25% of the patients had no residual viable tumors at histopathological examination of the liver explant, and extensive tumor damage was achieved in 42.85% of our patients.

Yao FY *et al.* presented a prospective study in 2005 that addressed the value of pre-transplant locoregional treatment [20]. Relevant downstaging was achieved in their cohort in 70% of the patients, and complete tumor necrosis was achieved in 44% of the patients, which is superior to the results published by Majno PE *et al.*, Decaens T *et al.*, Zen C *et al.* and to our own results [9, 10, 18]. Decaens T *et al.* evaluated two groups separately, the TACE group and the untreated control group, both consisting of 100 patients. They achieved a total or at least subtotal necrosis in 30% of the patients [9].

The study of Morisco *et al.* [16] showed that the degree of injury varies between tumors showing different pattern of growth. Patterns 1 and 2 (characterized by a mass composed of multiple small tumor nodules separated by fibrous septa and a single large mass respectively) showed a lesser degree of necrosis compared to those tumor characterized by a single mass with satellite tumor nodules (pattern 3). Our present study came to the same conclusion, considering that more than 90% tumor damage was found in 67% of the pattern 3 nodules, comparing with just 14.29% and 40% in patterns 1 and 2 nodules respectively.

Of note, the only two tumors recurring after transplantation in our series were those with showed more than 90% of tumor damage and a pattern 3. This

association needs to be tested further in larger series, but raises questions about the pathogenesis of this phenomenon. The exact nature of the satellite tumor nodules in pattern 3 remains uncertain, and in particular whether it represent spread of tumor cells from the main mass into adjacent tissue or separate foci as part of field change. Tumor spread from the main mass either *via* direct infiltration or vascular invasion would fit well with post transplant recurrence, as tumor cells would have had access to the blood circulation at the time of transplantation. Whether infiltration occurs before TACE or as an effect of tumor destruction cannot be established. The hypothesis of peritumoral field change would be in agreement with the dominant tumor inhibition loss concept [21]. According to this concept, multiple foci of neoplastic cells may appear independently in a relatively small area, one of the nodules being able to grow faster than the others and secrete growth factor inhibitors. Through a paracrine-signaling manner, these molecules could limit the expansion of the adjacent nodules. If the tumor is only partially necrotic (pattern 2), it might be able to produce a sufficient amount of inhibiting factors in order to stop the expansion of adjacent tumor foci, but when the dominant tumor undergoes complete necrosis, the adjacent foci may exit the inhibition state. These presumed dormant tumors seem also to expand fast, considering that in one of our patients, the time interval between TACE and the transplant was only 59 days. Morisco F *et al.* had another point in favor of this sequence of events, stating that mitotic activity was found to be significantly higher in the HCC nodules in patients treated before transplantation, which had more necrosis [16].

Thus, pattern 3 nodules might become more aggressive after TACE and selection of resistant clones from the adjacent satellite nodules may give rise to recurrence or to distant metastasis. Further studies are needed to validate that hypothesis.

Response to TACE is assessed clinically by identifying variation in the characteristics of the tumor appearance. The *European Association for the Study of the Liver* (EASL) recommended the use of contrast enhancement on CT as the standard modality to determine the tumoral response to treatment. Areas of tumor enhancement were to be considered viable whereas non-enhancing regions reflected tissue necrosis. The EASL also stated that tumor size measurements might not be accurate because they would not take into account the true extent of tumor necrosis [22]. The *American Association for the Study of Liver Disease* HCC practice guidelines [23, 24] recommend using a decrease in the concentration of tumor markers, the identification of large intra-tumoral necrotic areas and reduction in tumor burden in dynamic CT or MRI [22]. Contrast-enhanced MRI uses an extracellular contrast agent to determine areas of tumor enhancement. The enhanced portions of the tumor are considered viable, whereas non-enhancing portions are presumed to be necrotic. The disadvantage of this method is the inability to distinguish viable tissue from reactive granulation tissue [19]. This fits with the histological observation that areas of non-viable tumor consist of a combination

of necrosis, fibrous scarring and granulation tissue, and that the effect of treatment on the tumor mass is not simply of reduction in its total volume, or cumulative surface as assessed bidimensionally on macroscopic slices or tissue sections. In addition, our two cases of recurrent HCC post-transplantation had a very limited amount of viable tumor at transplantation, confirming that just the percentage of necrotic tumor is not a sufficient criterion of treatment response.

Histological assessment of tumor response has many limitations, even on specimens derived from surgical resections or transplantation. The tumor mass can be sectioned into thin slices, particularly after fixation and with use of metal bars to guide the scalpel or knife blade. In our experience, a full-face slice is achieved with difficulty even despite good fixation and the use of guide bars, as the necrotic tissue is brittle and tends not to adhere to rest of the tissue section. Even a thin, few mm thickness slice, is still of a much larger order of magnitude compared to the microscopic fields in which the relationships between residual tumor and the post-TACE changes are assessed. Another limitation is to represent histologically the full tumor face. This can be achieved for tumors of round or oval shape in which the transverse diameter is up to about 20 mm. Multiple full face cross sections of the tumor could therefore be submitted individually in single conventional embedding cassettes which, despite the limitation related to the section thickness described above, would give a reasonable representation of the entire tumor surface. In such cases assuming the cut thickness is known and remains constant, and the histological representation of viable and non-viable tumor is maintained throughout the section thickness, it would be possible to calculate the tumor mass volume, and the proportional volumetry of viable and non-viable tumor, or even necrosis, fibrous scarring, granulation tissue and various areas of viable tumor differentiation, perhaps with the aid of 3D reconstruction software. A similar approach would be impractical for larger tumors, as whole tumor embedding would involve a large number of conventional tissue blocks, unless large “megablocks” are used.

Tissue shrinkage and expansion after fixation and the following stages of tissue preparation are also a limitation to precise measurement and surface area calculation and volumetry.

Another aspect to take into account is the morphological definition of necrosis. Post-TACE HCC necrosis usually appears to the pathologist eyes as large area of tumor appearing as homogenous sheets of amorphous eosinophilic material, with cell ghosts in the background and often a retained reticulin and sinusoidal structure, which can be highlighted by a silver impregnation stain or immunohistochemistry for endothelial markers. The true state of morphologically “viable” tissue interspersed between areas of necrosis or in the vicinity however remains usually unknown. A previous study has show that peritumoral areas appearing as viable can be actually defunct when histochemical techniques are applied to test enzymatic function [25].

The response of tumor to therapy could also consist of differentiation into a mature phenotype simulating the normal non-neoplastic counterpart. This has been

observed in hepatoblastoma [26], and in a mouse model of HCC regression and progression following c-myc activation/inactivation [27]. In other words, tissue, which appears non-neoplastic and or viable, could be dead and/or neoplastic in various combinations.

Local ablation therapy is now being used for other sites besides liver, for primary or metastatic cancer, including lung and kidney. Most of the literature that we were able to access in relation to this topic is clinically orientated and does not give much information on the histological aspects of tissue response to treatment. No other histological changes of the tumors, apart from the ones associated with coagulative necrosis, have been reported on biopsy or resection specimens [28–30].

☞ Conclusions

Our study on post-TACE HCC raises the following points.

Extensive tumor destruction was achieved in 42.85% of the patients, with complete destruction in 25% of them, broadly replicating what described in the literature.

The percentage of necrosis is not related to the total cumulative surface of the nodule, so other tumor characteristics might be of interest in respect to response to TACE.

We did not establish any correlation between the proportion of viable tumor and time interval between TACE and transplantation due to a small number of cases and an individualized and variable number of TACE cycles in each case.

Most of the post-TACE viable tumors were moderately differentiated, but there were also biphasic tumors, as a mixture of moderately differentiated with well or poorly differentiated tumors. Fourteen percent of them showed a mixed hepatocholangiocellular phenotype, observation which supports the idea of a phenotypical-differentiation associated with TACE. As described particularly in the context of hepatoblastoma, it cannot be excluded that at least part of the tumor, which has survived to the toxic-ischemic insult, has differentiated into normal appearing hepatic tissue.

The degree of tumor damage could vary depending on the tumor growth pattern, whether as a single mass, a single mass with satellite nodules or a conglomerate of small nodules; this could explain why tumors with almost complete necrosis after TACE can recur after transplantation.

Treatment response cannot be based solely on change in tumor size, and changes in tumor contrast enhancement by imaging are due to necrosis and post-necrotic changes.

Tissue appearing as viable histological sections could be biologically dead. Assessment of tumor response to treatment should ideally be based on methods of analysis involving for example enzymatic function.

Despite the limitations of histology, image analysis and possibly 3D reconstruction, could assists in investigating post-treatment tumor structure and its changes.

Contribution Note

The first two authors have equally contributed to this work.

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