**Hepatoxicity by combination treatment of temozolomide, artesunate and Chinese herbs in a glioblastoma multiforme patient.**

**Case report and review of the literature**

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**Abbreviations:** AGT, O6-alkylguanine-DNA alkyltransferase; ARS, artemisinin; ART, artesunate; BER, DNA base-excision repair; CNS, central nervous system; DEX, dexamethasone; DSB, double strand break; GBM, glioblastoma multiforme; GM-CSF, granulocyte-monocyte colony stimulating factor; GST, glutathione S-transferase; INF, interferon; HR, homologous recombination; MGMT, methyl-guanine methyl transferase; MMR, DNA mismatch repair; MTX, methotrexate; NHEJ, non-homologous end-joining; TMZ, temozolomide; VCR, vincristine

**Abstract**

Glioblastoma multiforme (GBM) represents an aggressive tumor type with poor prognosis. The majority of GBM patients cannot be cured. There is high willingness among patients for the compassionate use of non-approved medications, which might occasionally lead to profound toxicity. A 65 year-old patient with glioblastoma multiforme (GBM) has been treated with radiochemotherapy including temozolomide (TMZ) after surgery. The treatment outcome was evaluated as stable disease with a tendency to slow tumor progression. In addition to standard medication (ondansetron, valproic acid, levetiracetam, lorazepam, clobazam), the patient took the antimalarial drug artesunate (ART) and a decoction of Chinese herbs (*Coptis chinensis, Siegesbeckia orientalis, Artemisia scoparia, Dictamnus dasycarpus*). In consequence, the clinical status deteriorated. Elevated liver enzymes were noted with peak values of 238 U/L (GPT/ALAT), 226 U/L (GOT/ASAT), and 347 U/L (γ-GT), respectively. After cessation of ART and Chinese herbs, the values returned back to normal and the patient felt well again. In the literature, hepatotoxicity is well documented for TMZ, but is very rare for ART. Among the Chinese herbs used, *Dictamnus dasycarpus* has been reported to induce liver injury. Additional medication included valproic acid and levetiracetam, which are also reported to exert hepatotoxicity. While all drugs alone may bear a minor risk for hepatotoxicity, the combination treatment might have caused increased liver enzyme activities. It can be speculated that the combination of these drugs caused liver injury. The combinational use of TMZ, ART plus Chinese herbs outside of clinical trials cannot be recommended for GBM treatment.

We conclude that standard radiochemotherapy with TMZ together with the compassionate use of ART and Chinese herbs is not recommended for GBM.

**Introduction**

Glioblastoma multiforme (GBM) represents the most common and most aggressive brain tumor and has a poor prognosis ([Woehrer et al. 2014](#_ENREF_106)). The etiology of GBM is not completely understood. Exposure to ionizing radiation, hereditary factors (Li-Fraumeni syndrome, neurofibromatosis), malaria as well as viral infections (herpes simplex virus-6, human cytomegalovirus) have been discussed as possible risk factors ([Crawford et al. 2009](#_ENREF_20); [Dziurzynski et al. 2012](#_ENREF_27); [Lehrer 2010](#_ENREF_64); [Madden et al. 2010](#_ENREF_69); [Ostrom et al. 2014](#_ENREF_82)).

In addition to surgery, the current GBM therapy is based on temozolomide (TMZ) combined with radiotherapy, which significantly improves progression-free and overall survival times compared to radiotherapy alone ([Nagasawa et al. 2012](#_ENREF_76); [Stupp et al. 2009](#_ENREF_97); [Yang et al. 2014a](#_ENREF_108)). TMZ has been approved to treat GBM and metastatic melanoma. It preferentially methylates and alkylates DNA at N7 and O6 positions of guanine residues. TMZ is a prodrug, which is spontaneously activated in aqueous solution to the dacarbazine metabolite, 5-(3-methyl-1-triazeno)imidazole-4-carboxamide) ([Bei et al. 2010](#_ENREF_9)). This reaction can take place without involvement of hepatic cytochrome P450 monooxygenase isozymes. DNA O6-methylguanine adducts induced by TMZ are repaired by O6-alkylguanine-DNA alkyltransferase (AGT), also termed methyl-guanine methyl transferase (MGMT). High expression of AGT/MGMT confers resistance to TMZ, whereas methylation of the MGMT promoter, which causes downregulation of MGMT expression, is associated to sensitivity to this drug ([Berghoff et al. 2015](#_ENREF_12)). Two other DNA repair mechanisms also contribute to TMZ resistance: mismatch repair (MMR) and base excision repair (BER). MMR recognizes base mismatches and insertion-deletion loops in DNA, while BER repairs TMZ-induced N-based guanine lesions ([Marchesi et al. 2007](#_ENREF_70)).

As the majority of GBM patients cannot be cured, there is a desperate quest for novel treatment options in neuro-oncology, and the willingness for compassionate use of non-approved medications is high at the side of the patients. In addition to immunological and gene therapeutic approaches ([Wang et al. 2015](#_ENREF_102); [Zhai et al. 2015](#_ENREF_112)), phytochemicals might be of some interest as candidates for GBM treatment ([Kuete et al. 2015](#_ENREF_59); [Kuete et al. 2014](#_ENREF_60); [Pistollato et al. 2015](#_ENREF_84)).

In addition to their antimalarial activity, artemisinin (ARS) from the Chinese medicinal herb, *Artemisia annua* L. (Asteraceae) and its derivatives (artesunate, artemether, dehydroartemisinin) also exert remarkable cytotoxic effects towards a wide spectrum of tumor cell lines ([Efferth et al. 2001](#_ENREF_30); [Efferth et al. 2002](#_ENREF_32); [Efferth et al. 1996](#_ENREF_34); [Efferth et al. 2003a](#_ENREF_35)). Artemisinin (ARS)-type drugs are also active against diverse syngeneic animal tumors ([Disbrow et al. 2005](#_ENREF_24); [Lai and Singh 2006](#_ENREF_62); [Moore et al. 1995](#_ENREF_74)) and human xenograft tumors in nude mice ([Dell'Eva et al. 2004](#_ENREF_23); [Du et al. 2010](#_ENREF_26); [Li et al. 2007](#_ENREF_65); [Ma et al. 2011](#_ENREF_68)). Activity was also reported against brain tumor cells *in vitro* and *in vivo* ([Berdelle et al. 2011](#_ENREF_10); [Cao et al. 2014](#_ENREF_14); [Chen et al. 2015](#_ENREF_16); [Efferth et al. 2004b](#_ENREF_33); [Huang et al. 2007](#_ENREF_49); [Wu et al. 2009](#_ENREF_107)). Compassionate uses of ARS-type drugs and *Artemisia annua* preparations for cancer therapy of veterinary and human tumors encouraged the performance of several pilot Phase I/II trials in cancer patients ([Berger et al. 2005](#_ENREF_11); [Breuer and Efferth 2014](#_ENREF_13); [Jansen et al. 2011](#_ENREF_50); [Krishna et al. 2015](#_ENREF_58); [Rutteman et al. 2013](#_ENREF_92); [Singh and Verma 2002](#_ENREF_94); [Zhang et al. 2008](#_ENREF_114)).

Synergistic interactions between TMZ and artesunate (ART) ([Huang et al. 2008](#_ENREF_48); [Karpel-Massler et al. 2014](#_ENREF_51); [Zhang et al. 2015](#_ENREF_113)) as well as between ionizing radiation and ART ([Kim et al. 2006](#_ENREF_55); [Reichert et al. 2012](#_ENREF_89)) raised some interest in ART for compassionate use in GBM patients. Here, we report on the hepatotoxic reaction of the combined treatment of TMZ and ART in a GBM patient, which indicates that the synergism between TMZ and ART observed *in vitro* might not be limited to cancer cells, but might also harm healthy tissues.

**Case report**

A 65 year-old patient has been diagnosed with GBM WHO grade 4 on May 26th 2014. Two days later, the tumor in the left-central brain has been removed by navigation- and ultrasound-based microsurgery and intraoperative radiotherapy (IORT, 20 Gy) in the framework of the Intrago trial ([Giordano et al. 2014](#_ENREF_44)). A small satellite lesion in the left central region was inoperable. She then underwent radiochemotherapy with TMZ (50 mg/m2/d p.o.) and external-beam radiotherapy to a total dose of 60 Gy in 30 fractions. Four weeks after radiochemotherapy, cycling chemotherapy (5 days on, 23 days off) was initiated with 150 mg/m2/d/cycle for the first two cycles and 200 mg/m2/d/cycle for subsequent cycles ([Stupp et al. 2005](#_ENREF_98)). During radiochemotherapy and chemotherapy, concomitant ondansetron (8 mg/d) was given to prevent nausea.

Exon and transcriptome sequencing of tumor and normal tissue was performed at the National Center for Tumor Diseases (NCT, Heidelberg, Germany), which confirmed the histological diagnosis of GBM. In total, 65 nucleotide substitutions, three focal and 17 larger copy number changes were found. The promoter region of the *MGMT* gene was methylated, which supports chemotherapy with TMZ.

The patient also received anticonvulsive medication due to episodes symptomatic epilepsy (focal seizures), including levetiracetam (1,000 mg/d starting dose, escalated to 2,750 mg/d), lorazepam (1 mg/d p.r.n.) and clobazam (5 mg/d p.r.n.).

Starting September 20th 2015, the patient voluntarily decided to additionally start off-label treatment with ART capsules (200 mg/d) and *Coptis-Kush* decoct (0,5 g Rhizoma Coptidis chinensis; 3,0 g Herba Siegesbeckiae orientalis; 6,0 g Herba Artemisiae scopariae H; 2 g Radix Dictamni dasycarpi; 20 doses). *Coptis-Kush* intake was stopped 2.5 weeks later and ART intake on October 20th 2015.

As outlined in **Table 1**, liver enzyme activities increased after intake of ART and *Coptis-Kush*. Leukocyte counts and especially monocytes were continuously decreased since August 2015 and remained below the normal range even after cessation of ART/*Coptis-Kush*, indicating that this leukopenia may be unrelated to ART/*Coptis-Kush.*

Symptoms appearing at that time were weight loss, heartburn, left-thoracic sting independent of stress, nausea, adynamia, fatigue, and depressive mood. After ceasing ART and *Coptis-Kush* intake liver parameters gradually returned back to normal. Presently, the patient feels well and all symptoms vanished.

Of note, over the whole episode of hepatic enzyme elevation and subsequent clinical deterioration, serial MRIs revealed no signs of progressive disease regarding the brain tumor. Remarkably, the tumor did not progress even one year after intake of ART.

**Discussion**

Here, we report a case of hepatotoxicity in a GBM patient treated with TMZ, ART and Chinese herbs. It is unclear, whether one of these two drugs alone are responsible for this toxic reaction or whether it developed only by their combination. However, the evident hepatic enzyme elevation and clinical deterioration immediately after initiation of treatment suggests that ART and/or Chinese herbs might have been at least eliciting events.

TMZ’s tolerability represents a considerable concern for its use. Myelotoxicity is a well-known severe adverse effect of TMZ (anemia, drop of hemoglobin levels, neutropenia, thrombocytopenia). Further side effects include nausea, vomiting, loss of appetite, constipation, convulsions, diarrhea, skin rash, fatigue, weakness, dizziness, blurred vision, insomnia, headache, and very rarely, alopezia. Hepatotoxicity did not play a significant role during official approval as cancer drug. During the postmarketing phase, however, numerous cases with severe liver injury have been reported (**Table 2**) (for review see also [Dixit et al. 2011](#_ENREF_25)). The range of toxic reactions ranged from elevation of liver transaminases to jaundice, cholestatic hepatitis, steatohepatitis, hepatic encephalopathy, and hepatitis B virus reactivation (**Table 2**).

TMZ reactivates hepatitis B viruses ([Fujimoto et al. 2012](#_ENREF_38); [Purchiaroni et al. 2014](#_ENREF_88)). Furthermore, prior malaria infections might increase the risk of GMB development ([Lehrer 2010](#_ENREF_64)). The patient presented here experienced several malaria infections many years ago. It is unknown, whether TMZ reactivates or stimulates a still unknown, carcinogenic virus transmitted by *Anopheles* mosquitos that also contribute to hepatotoxicity.

ART seems to be a rather safe drug. Large clinical studies and meta-analyses with many thousands of malaria patients did not show serious side effects, although there is a paucity of large-scale clinical trials suitable to detect rare, but significant toxicity ([Efferth and Kaina 2010](#_ENREF_31)). Toxicity studies *in vitro* and *in vivo* (mice, rats, rabbits, dogs, monkeys) provided hints for potential neurotoxicity, embryotoxicity, genotoxicity, hemato- and immunotoxicity, cardiotoxicity, nephrotoxicity, and allergic reactions. Long-term availability rather than short-term peak concentrations of artemisinins may cause toxicity. Rapid elimination of artemisinins after oral intake represents a relatively safe route of administration compared to delayed drug release after intramuscular injection. This explains why considerable toxicities were found in the majority of animal experiments, but not in human studies. Moreover, there are drug-related differences, *i.e*., intramuscular application of artemether or arteether bears some toxic potential, but not ART, which is safe and gives good profiles after i.m. administration in severe malaria. This fact may be important determining dose-limiting toxicities for cancer.

Large meta-analyses with many thousands of malaria patients reported elevated liver enzymes in 0.9% of all cases ([Ribeiro and Olliaro 1998](#_ENREF_90)). Severe liver injury was reported in two cases after consumption of ARS-containing herbal supplements ([CDC 2009](#_ENREF_15); [Kumar 2015](#_ENREF_61)). According to the current literature, the hepatotoxic risk is much higher for TMZ than for ART. This conclusion is also supported by animal experiments, which described liver enzyme changes upon treatment with ARS-based drugs, but no severe acute liver injuries (**Table 3**) ([Berger et al. 2005](#_ENREF_11); [Breuer and Efferth 2014](#_ENREF_13); [Ericsson et al. 2014a](#_ENREF_36); [Jansen et al. 2011](#_ENREF_50); [Michaelsen et al. 2015](#_ENREF_72); [Rutteman et al. 2013](#_ENREF_92); [Singh and Verma 2002](#_ENREF_94); [Zhang et al. 2008](#_ENREF_114)).

Hepatotoxicity represents a rare event upon combined treatment with TMZ or ART, although more cases of hepatotoxicity are documented for TMZ than for ART. It is possible that drug-drug interaction may synergistically increase the low hepatotoxic potential of both drugs applied alone. Hepatotoxicity by ART is extremely rare in malaria, but occurred in the present case of GBM. This might be due to the fact that concentrations of ART used for malaria treatment (usually 50 mg/day for 3-4 days) are lower than those used for cancer therapy (in the present case: 200 mg/d for four weeks). Higher ART doses may increase the risk for toxic reactions of the body.

While the exact reasons for the hepatotoxicity of the TMZ/ART combination in the patient presented here are unknown, several mechanisms might have account for this hepatotoxic reaction:

(1) Drugs affect the activity of drug metabolizing phase I and II enzymes, *e.g.* cytochrome P450 monooxygenases (CYPs) and gluthathione S-transferases (GSTs).

(2) Gene polymorphisms increase drug activity towards tumors and toxicity towards normal tissues.

(3) A drug combination regimen damages DNA at different sites leading to increased DNA damage in normal organs.

Although the activation of TMZ as prodrug to the active metabolite occurs spontaneously in aqueous solution, additional metabolization by CYPs in the liver may be possible. A recent study reports on factors associated with severe hematological toxicity of GBM patients treated with standard therapy (TMZ + radiation) ([Lombardi et al. 2015](#_ENREF_67)). Among different other factors, polymorphisms in *CYP* genes predicted severe myelotoxicity. It can be speculated that CYP polymorphisms also increase the hepatotoxic potential of TMZ in these patients. Polymorphisms in other genes (*MGMT, NQO1, GSTP1*) were related to an increased risk of myelotoxicity in a subset of GBM patients ([Armstrong et al. 2009](#_ENREF_6)). It can be speculated that specific gene polymorphisms also contribute to TMZ-induced hepatotoxicity. An association of TMZ-induced toxicity to DNA damage and repair has not been reported as of yet.

 In contrast to TMZ, ARS derivatives are metabolized in the liver to the active metabolite, dihydroartemisinin. ARS, ART, artemether and dihydroartemisinin inhibit the enzymatic activity of several recombinant CYP enzymes (CYP1A2, 2B6, 2C19, and 3A4) ([Ericsson et al. 2014b](#_ENREF_37)). A high risk of interaction *in vivo* could be predicted, if ARS-type drugs are co-administered with other drugs that are CYP1A2 or 2C19 substrates. Subjects with CYP2AG\*1B, which is responsible for ultra-rapid ART metabolism revealed more adverse drug reactions including elevated liver enzymes ([Yusof and Hua 2012](#_ENREF_110)).

Microarray analyses of tumor cell lines revealed that the expression of several DNA damage response and repair genes significantly correlated with cellular response of ARS-type drugs in these cell lines, *e.g. ERCC5, FEN1, HMG1, HMF17, LIG1, RPS3, UNG,* and *UBE2A* ([Efferth et al. 2002](#_ENREF_32); [Efferth et al. 2003a](#_ENREF_35)). ART may induce DNA damage by cleaving its endoperoxide moiety, which leads to reactive oxygen species- or carbon-centered radical-mediated DNA damage. ART dose-dependently induced DNA breaks in the comet assay and induced the expression of γ-H2AX, which is considered as marker for DNA double-strand breaks ([Li et al. 2008](#_ENREF_66)). Polymerase β-deficient cells were more sensitive towards ART than wild-type cells, indicating that DNA lesions are repaired by BER. Irs1 and VC8 cells defective in homologous recombination (HR) by XRCC2 and BRCA2 inactivation, as well as XR-V15B cells defective in non-homologous end-joining (NHEJ) by Ku80 inactivation of Ku80 were also more susceptible to ART than the corresponding wild-types. ([Li et al. 2008](#_ENREF_66)).

ART is a powerful inducer of oxidative DNA damage. It generates formamidopyrimidine DNA glycosylase-sensitive sites and forms 8-oxoguanine and 1,N6-ethenoadenine. In human LN-229 glioblastoma, ART-induced oxidative DNA damage was attenuated by the radical scavenger, N-acetyl cysteine. Oxidative DNA damage caused DSBs as determined by γ-H2AX foci. Knockdown of Rad51 by siRNA and inactivation of DNA-PK strongly sensitized glioma cells to ART. These data also indicate that both HJ and NHEJ pathways contribute to ART-induced DSB repair. ART provoked DNA damage response that was characterized by phosphorylation of ATM, ATR, Chk1, and Chk2 ([Berdelle et al. 2011](#_ENREF_10)). Our initial findings on ART-induced DNA damage were confirmed for other artemisinin derivatives ([Alcantara et al. 2013](#_ENREF_2); [Park et al. 2015](#_ENREF_83)).

 The DNA damaging properties of ARS-type drugs may also play a role for their toxicity towards normal cells. In mice, ART caused DNA damage in liver cells and spermatozoa. It affected the antioxidant stress response (depletion of glutathione, inhibition of superoxide dismutase and increase of lipid peroxidation) ([Aquino et al. 2011](#_ENREF_4); [Aquino et al. 2013](#_ENREF_5); [Singh et al. 2015](#_ENREF_95)). These data speak for potential genotoxicity *in vivo*.

**Conclusions and perspectives**

The compassionate GBM therapy by TMZ, ART and Chinese herbs is not recommended, as indicated by the present case of hepatotoxicity. ARS-type drugs confer ferrous iron-mediated oxidative damage leading to DNA damage in tumor cells ([Efferth 2015](#_ENREF_28); [Efferth et al. 2004a](#_ENREF_29); [Kelter et al. 2007](#_ENREF_54); [Ooko et al. 2015](#_ENREF_81)). As the liver is a major iron-storing organ in the human body, hepatotoxicity by ARS and its derivatives may be a special concern. In a pilot I/II trial, we reported that ART monotherapy is safe and efficient in cervix carcinoma ([Jansen et al. 2011](#_ENREF_50)). ART plus surgery was also safe and efficient to treat colon carcinoma ([Krishna et al. 2015](#_ENREF_58)). In another Phase I trial with veterinary tumors, we found several side effects (fever, anemia, thrombocytopenia, and gastrointestinal toxicity), but not hepatotoxicity ([Rutteman et al. 2013](#_ENREF_92)).

While no reports on the hepatotoxicity of *Coptis chinensis* have been published, this herb may rather act in a hepatoprotective manner ([Choi et al. 2013](#_ENREF_18)). The same is true for *Artemisia scoparia* ([Gilani and Janbaz 1993](#_ENREF_42); [1994](#_ENREF_43); [Wang et al. 2013b](#_ENREF_104)). Although these two drugs have been reported to be hepatoprotective, they did not prevent the increase of hepatic enzyme activities in the GBM patient presented here, which raises the question about their activity. Nothing is known about the potential liver toxicity of *Siegesbeckia*. However, *Dicramnus dasycarpus* induces liver injury ([Lee et al. 2015](#_ENREF_63)). Therefore, it cannot be excluded that this plant contributed to the hepatotoxicity described in the present case report. Even the other medications used in GBM management could be considered for their toxic potential. Valproic acid and Levetiracetam (lacosamide) have been also reported to exert hepatotoxic effects ([Gerstner et al. 2008](#_ENREF_41); [Sunwoo et al. 2015](#_ENREF_99)).

In addition to TMZ and ART, the patient ingested a variety of herbs and, moreover, multiple antiepileptic and sedative drugs. As many of these drugs are known to be hepatotoxic, the appearance of fulminant hepatotoxicity is not highly surprising and it is difficult to dissect the contribution of the different medications for the outbreak of acute liver toxicity. Compassionate intake of such complex additional medications together with anticancer drugs can be expected to cause severe side effects not only in GBM patients, but also in patients treated with different therapeutic regimen than TMZ. Controlled clinical trials have to clarify, how frequently hepatotoxicity may occur upon GBM treatment with combinations of TMZ, ART and other herbal drugs. This case of severe hepatotoxicity also illustrates that unexpected toxicity can occur not only with non-approved drugs, but even with novel combinations of clinically established drugs.

In conclusion, the presented case can be taken as a hint for the possible consequences of compassionate use of non-approved drugs or unproven drug combinations. Drug therapy should always be in accordance to the guidelines of good clinical practice and new therapies or combinations evaluated within controlled clinical trials. Concepts to apply marketed and otherwise safe drugs used for other non-cancer indications for GBM therapy should be handled with care to avoid unexpected severe toxicities ([Kast et al. 2013](#_ENREF_52); [Kast et al. 2014](#_ENREF_53)).

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**Table 1:** Blood count and clinical chemistry.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Units** | **Reference area** | **April 8th 2015** | **August 10th 2015** | **September 28th 2015** | **October 12th 2015** | **October 16th 2015** | **October 21st 2015** | **October 26th 2015** | **October 29th 2015** | **October 29th 2015** | **November 2nd 2015** | **November 9th 2015** | **Novemer 16th 2015** | **November 30th 2015** | **December 9th 2014** | **December 16th 2015** | **December 28th 2015** | **January 11th 2016** | **January 25th 2016** | **January 28th 2016** | **February 2nd 2016** |
| **Hematology:** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Erythrocyte sedimentation rate | mm/h | ≤25 | 20 | 7 | 11 |  |  | 21 | **39** | **33** |  | **35** | 21 | 15 | 12 | 9 | 10 |  |  |  |  |  |
| Leucocytes | count/nL | 3.7-10.1 | 6.2 | **2.5** | **3.1** | **2.2** | **2.15** | **2.8** | **2.0** | **2.3** | **3.02** | **2.3** | **2.2** | **2.7** | **3.1** | **2.9** | **2.7** | **3.18** | **3.01** | **2.28** | **2.73** | **2.97** |
| Neutrophiles | % | 42-76 | **82** | 64 | 64 | 52 | 51 | 62 | 50 | 61 |  | 64 | 55 | 57 | 68 | 66 | 67 |  |  |  |  |  |
| Lymphocytes | % | 18-45 | **9** | 18 | 20 | 23 | 23 | 18 | 28 | 24 |  | 20 | 29 | 28 | 18 | **17** | **19** |  |  |  |  |  |
| Monocytes | % | 3-10 | 7 | **14** | **11** | **20** | **24** | **16** | **16** | 10 |  | **12** | **11** | 10 | 9 | 14 | 10 |  |  |  |  |  |
| Eosinophiles | % | 1-7 | 1 | 2 | 4 | 4 | 1 | 3 | 5 | 3 |  | 3 | 3 | 3 | 3 | 2 | 3 |  |  |  |  |  |
| Basophiles | % | ≤2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 |  | 1 | 2 | 2 | 2 | 1 | 1 |  |  |  |  |  |
| Erythrocytes | count/pL | 3.8-5.0 | 4.5 | 4.5 | 4.7 | 4.5 | 4.36 | 4.6 | 4.1 | 4.0 | 4.0 | 4.1 | 4.2 | 4.2 | **4.3** | 4.3 | 4.4 | 4.28 | 4.60 | 4.66 | 4.63 | 4.76 |
| Hemoglobin | g/dL | 11.6-15.1 | 13.6 | 13.9 | 14.5 | 13.5 | 12.9 | 13.5 | 12 | 11.7 | 11.8 | 12.2 | 12.7 | 13.1 | 13.2 | 13.2 | 13.4 | 13.3 | 14.2 | 14.2 | 14.1 | 14.4 |
| Hematokrit | L/L | 0.34-0.44 | 0.41 | 0.42 | 0.44 | 0.39 | 0.37 | 0.39 | 0.35 | 0.34 | 0.35 | 0.37 | 0.39 | 0.39 | 0.40 | 0.4 | 0.40 | 0.38 | 0.42 | 41.3 | 41.3 | 41.7 |
| MCV | fL | 81-99 | 92 | 93 | 93 | 86 | 85 | 85 | 85 | 87 | 86.8 | 89 | 92 | 92 | 94 | 93 | 91 | 87.6 | 90.4 | 88.6 | 89.2 | 87.6 |
| MCH | pg | 27-34 | 30 | 31 | 31 | 30 |  | 29 | 29 | 30 | 29.5 | 30 | 30 | 31 | 31 | 31 | 30 | 31.1 | 30.9 | 30.5 | 30.5 | 30.3 |
| MCHC | g/dL | 32-36 | 33 | 33 | 33 | 35 | 34.7 | 35 | 35 | 34 | 34 | 33 | 33 | 34 | 33 | 33 | 33 | 35.5 | 34.1 | 34.4 | 34.1 | 34.5 |
| Thrombocytes | count/nL | 150-361 | 248 | 223 | 179 | 261 | 249 | 209 | 218 | 304 | 310 | 353 | 290 | 246 | 281 | 243 | 239 | 172 | 285 | 174 | **146** | 204 |
| **Clinical Chemistry:** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| GPT (ALAT) | U/L | 0-35 | 17 | 16 | 157 | **191** | **223** | **238** | **116** | **82** | **80** | **67** | **45** | 35 | 26 | 26 | 27 | 23 | 26 | 22 | 22 | 24 |
| GOT (ASAT) | U/L | 0-35 | 18 | 21 | 88 | **102** | **138** | **226** | **41** | **38** | **40** | 35 | 27 | 24 | 21 | 26 | 33 | 23 | 35 | 18 | 17 | 20 |
| γ-GT | U/L | 0-40 | 26 | 22 | 29 | **94** | **196** | **313** | **347** | **285** | **295** | **241** | **170** | **125** | **76** | **58** | **51** | 40 | **42** | 32 | 32 | 31 |
| Lactate dehydro-genase (LDH) | U/L | ≤214 | 198 | 177 | 210 | **225** | **223** | **280** | 191 | 196 |  | 196 | 180 | 173 | 207 | 172 | 162 |  |  |  |  |  |
| Choline esterase | kU/L | 4.3-11.3 | 9.6 | 8.9 | 9.4 | 6.9 |  | 7.1 | 6.7 | 7.4 |  | 8.5 | 8.6 | 8.9 | 9.3 | 8.7 | 8.4 |  |  |  |  |  |
| Creatinin  | mg/dL | ≤1.0 | 1.0 | 1.0 | 1.0 | 0.9 | 0.85 | 0.9 | 0.8 | 0.9 |  | 0.9 | 0.9 | 0.9 | 0.8 | 0.9 | 0.8 | 0.73 | 0.88 | 0.88 | 0.74 | 0.73 |
| Glomerular filtration rate (CKD-EPI) | mL/min |  | 59 | 59 | 58 | 66 | 71 | 66 | 77 | 66 | 75 | 66 | 66 | 66 | 76 | 66 | 76 | 85 | 68 | 68 | 84 | 85 |
| urea  | mg/dL | 17-49 | 28 | 32 | 39 | 48 | 40 | 36 | 32 | 23 |  | 33 | 30 | 27 | 28 | 29 | 30 | 29 | 35 | 34 |  |  |
| uric acid | mg/dL | 0-5.7 | 4.6 | 4.2 | 4.4 | 3.5 | 3.2 | 3.8 | 4.0 | 3.8 |  | 4.3 | 3.8 | 4.0 | 4.1 | 4.3 | 3.5 | 3.7 | 3.8 | 3.6 |  |  |
| C-reactive protein (CRP) | mg/L | ≤5 | 2.8 | <1.0 | 1.7 | 1.3 |  | 1.6 | **6.0** | <1.0 | <1.0 | <1.0 | <1.0 |  |  |  |  |  |  |  |
| Triglycerides | mg/dL | ≤150 | 50 | 65 | 45 | 73 |  | 114 | 78 | 80 |  | 69 | 66 | 63 | 47 | 40 | 65 |  |  |  |  |  |
| Cholesterol, total | mg/dL | ≤200 | 199 | **208** | **231** | 155 |  | **220** | **242** | **250** |  | **259** | **265** | **267** | **243** | **239** | **227** |  |  |  |  |  |
| HDL choleterol | mg/dL | ≥45 | 71 |  | 96 | 56 |  | 51 | 62 | 67 |  | 78 | 87 | 84 | 85 | 91 | 72 |  |  |  |  |  |
| LDL cholesterol | mg/dL | ≤160 | 116 | 119 | 121 | 82 |  | 149 | 151 | **161** |  | **175** | **169** | **165** | 154 | 141 | 143 |  |  |  |  |  |
| Atherosclerosis index (LDL/HDL) |  | <3 | 1.6 |  | 1.3 | 2.0 |  | 2.9 | 2.4 | 2.4 |  | 2.2 | 1.9 | 2.0 | 1.8 | 1.5 | 2.0 |  |  |  |  |  |
| Natrium | mmol/L | 133-146 | 142 | 145 | 142 | 142 | 142 | 142 | 143 | 142 |  | 142 | 142 | 144 | 143 | 141 | 143 |  |  |  |  |  |
| Potassium | mmol/L | 3.6-5.5 | 4.6 | 4.3 | **5.6** | **5.8** | 4.2 | 4.9 | 5.0 | 5.2 |  | 5.0 | 4.5 | 4.8 | 4.9 | 5.0 | 4.6 |  |  |  |  |  |
| Calcium | mmol/L | 2.1-2.6 | 2.36 | 2.38 | 2.35 | 2.29 | 2.20 | 2.27 | 2.27 | 2.35 | 2.28 | 2.34 | 2.35 | 2.37 | 2.40 | 2.31 |   |   |   |   |
| **Endocrinology:** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| TSH basal | µU/mL | 0.30-4.20 |  | 2.7 | 1.66 | 0.73 | 0.81 | 1.01 | 1.18 | 1.03 | 1.08 | 1.53 | 1.35 | 1.08 | 1.57 | 1.11 |   |   |   |   |

|  |
| --- |
| **bold number: below reference area** |
| **bold number in grey box : above reference area** |

**Table 2:** Literature survey on hepatotoxicity by temozolomide.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tumor type** | **Treatment regimen** | **No. of patients** | **Effect** | **Reference** |
| Metastatic melanoma | TMZ + interleukin-2 + GM-CSF | 74 | Transient liver function disturbances (grade 2 in 20, grade 3 in 15 patients) | ([de Gast et al. 2003](#_ENREF_21)) |
| Metastatic melanoma stage IV | TMZ + INF-α-2b | 47 | Elevation of liver function parameters | ([Richtig et al. 2004](#_ENREF_91)) |
| Metastatic melanoma | TMZ + INF-α-2b | 27 | Predominant non-hematological toxicity was hepatotoxicity G-III | ([Garcia et al. 2006](#_ENREF_39)) |
| GBM | TMZ | 1 | Hepatitis B reactivation | ([Chheda et al. 2007](#_ENREF_17)) |
| Brain metastasis of solid tumors | TMZ + radiotherapy + antiepileptic drugs | 33 | 45.5% hepatotoxicity (due to concomitantly administered antiepileptic drugs?) | ([Kouvaris et al. 2007](#_ENREF_57)) |
| GBM | TMZ + cilengitide | 1 | Acute cholestatic hepatitis with hepatic failure | ([Neyns et al. 2008](#_ENREF_77)) |
| GBM | TMZ + gefitinib | 26 | Increased liver transaminases as dose-limiting toxicity | ([Prados et al. 2008](#_ENREF_86)) |
| Melanoma stage III/IV | TMZ | 12 | Elevation of liver transaminases as dose-limiting toxicity | ([Bedikian et al. 2009](#_ENREF_8)) |
| GBM | TMZ + radiotherapy  | 85, 59 | 5 patients with elevated liver enzymes, 6 patients with elevated liver enzymes | ([Clarke et al. 2009](#_ENREF_19)) |
| GBM | TMZ + radiotherapy  | 38 | 3 patents with grade II increase of liver enzymes | ([Kong et al. 2010](#_ENREF_56)) |
| Relapsed childhood solid tumors | TMZ + VCR + irinotecan | 12 | 4 patents with dose-limiting hepatotoxicity | ([Wagner et al. 2010](#_ENREF_101)) |
| GBM | TMZ + radiotherapy  | 1 | Raised liver enzymes and bilirubin acute choleastasis and focal parenchymal liver inflammation | ([Dixit et al. 2011](#_ENREF_25)) |
| GBM | TMZ | 1 | Severe cholestatic liver damage and consecutive hepatic encephalopathy | ([Goldbecker et al. 2011](#_ENREF_45)) |
| GBM | TMZ + radiotherapy  | 46 | 2 patients with raised liver enzymes. One of them was in the healing phase of hepatitis A | ([Niewald et al. 2011](#_ENREF_78)) |
| GBM | TMZ + radiotherapy  | 1 | Raised liver enzymes, 630-fold raised hepatitis B virus DNA levels (virus reactivation) | ([Ohno et al. 2011](#_ENREF_79)) |
| Glioma | TMZ + radiotherapy  | 1 | Impaired liver function, acute liver failure, hepatitis B virus reactivation | ([Fujimoto et al. 2012](#_ENREF_38)) |
|  | TMZ + DEX | 1 | steatohepatitis | ([Miller et al. 2012](#_ENREF_73)) |
| Solid tumors | TMZ + bortezomib | 25 | Elevated hepatic enzymes as dose-limiting toxicity | ([Portnow et al. 2012](#_ENREF_85)) |
| GBM | TMZ (+ prior paracetamol) | 1 | severe sustained cholestatic hepatitis (possibly due to paracetamol) | ([Sarganas et al. 2012](#_ENREF_93)) ([Zamani and Mohammad Alizadeh 2012](#_ENREF_111)) |
| Anaplastic astro-cytoma, GBM | TMZ alone, TMZ + radiotherapy | 195, 178 | 30 patients with raised liver enzymes, 16 patients with raised liver enzymes | ([Wick et al. 2012](#_ENREF_105)) |
| Medulloblastoma | TMZ + bevacizumab + irinotecan | 9 | 1 patient with grade III elevation of liver function tests | ([Aguilera et al. 2013](#_ENREF_1)) |
| 3 brain tumors, 1 melanoma | TMZ | 4 | Idiosyncratic acute liver injury  | ([Grant et al. 2013](#_ENREF_46)) |
| CNS lymphoma | TMZ + nedaplatin + VCR + radiotherapy | 14 | 5 patients with abnormal liver functions | ([Wang et al. 2013a](#_ENREF_103)) |
| GBM | TMZ + radiotherapy | 21 | 3 patents with dose-limiting hepatotoxicity | ([Alonso-Basanta et al. 2014](#_ENREF_3)) |
| GBM | TMZ + radiotherapy | 1 | Transaminase elevation and severe jaundice symptoms; mixed-type hepatic/cholestatic hepatitis | ([Becker et al. 2014](#_ENREF_7)) |
| GBM | TMZ + radiotherapy + Chinese herbal formula | 1 | Severe liver toxicity with jaundice. After cessation of the herbal formula and TMZ liver enzymes normalized | ([Melchardt et al. 2014](#_ENREF_71)) |
| GBM | TMZ | 38 | fatal hepatic failure | ([Prescrire\_international 2014](#_ENREF_87)) |
| GBM | TMZ + radiotherapy + glucocorticoids | 1 | Acute hepatitis due to hepatitis B virus reactivation | ([Purchiaroni et al. 2014](#_ENREF_88)) |
| GBM | TMZ + radiotherapy | 1 | Irreversible cholestatic hepatitis | ([Sarganas et al. 2012](#_ENREF_93)) |
|  |  |  |  |  |
| Advanced glioma | TMZ-based regimens | 152 | No grade 3/4 liver toxicity | ([Yang et al. 2014b](#_ENREF_109)) |
| GBM | TMZ + radiotherapy | 1 | Severe liver injury | ([Grieco et al. 2015](#_ENREF_47)) |
| CNS lymphoma | TMZ + MTX | 48 | 21 patients with grade 3/4 liver toxicity | ([Omuro et al. 2015](#_ENREF_80)) |

Abbreviations: CNS, central nerval system; DEX, dexamethasone; GBM, glioblastoma multiforme; GM-CSF, granulocyte-monocyte colony stimulating factor; INF, interferon; MTX, methotrexate; TMZ, temozolomide; VCR, vincristine

**Table 3:** Literature survey on hepatotoxicity by artesunate.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Disease / condition** | **Treatment regime** | **No. of patients** | **Effect** | **Reference** |
| Liver cirrhosisMalaria | ARS ARS and derivatives | 19241 | Pharmacokinetics not different from healthy control subjectsNo serious adverse events or severe toxicity. Occasionally elevated liver enzymes (0.9%) | ([de Vries et al. 1997](#_ENREF_22))([Ribeiro and Olliaro 1998](#_ENREF_90)) |
| Healthy volunteers | ART/amodiaquine + efavirenz | 2 | Significantly increased hepatic transaminase levels | ([German et al. 2007](#_ENREF_40)) |
| Malaria | ART | 35 | No hepatotoxicity | ([Stepniewska et al. 2009](#_ENREF_96)) |
| Unclear protozoal infection | ARS-containing herbal supplement | 1 | Hepatitis | ([CDC 2009](#_ENREF_15)) |
| Healthy volunteers | ART + amodiaquine | 24 | Subjects with CYP2AG\*1B responsible for ultrarapid ART metabolism had more adverse drug reactions, including progression of liver enzymes | ([Yusof and Hua 2012](#_ENREF_110)) |
| Healthy volunteers | ART/Pyronaridine + metoprolol | 56 | Raised liver function tests in 5 patients | ([Morris et al. 2014](#_ENREF_75)) |
| Intake for general health maintenance | ARS-containing herbal supplement | 1 | Colestatic liver injury | ([Kumar 2015](#_ENREF_61)) |
| Severe and complicated malaria | Intravenous ART | 102 | Elevated hepatic enzyme levels (49%) | ([Twomey et al. 2015](#_ENREF_100)) |

Abbreviations: ARS, artemisinin; ART, artesunate