10.48047/jocaaa.2024.33.06.127

# Thioredoxin as a Novel Serological Biomarker in Alpha-Fetoprotein Negative Hepatocellular Carcinoma: Evidence and Mechanisms

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**Received:** 28 March 2024, **Accepted:** 17 April 2024, **Published:** 20 May 2024

## ABSTRACT

**Background:** Hepatocellular carcinoma (HCC) represents a global health challenge with high mortality, largely driven by late diagnosis. Alpha-fetoprotein (AFP), the conventional serological benchmark for HCC surveillance and diagnosis, exhibits suboptimal sensitivity and specificity, with a significant proportion of patients, up to 40%, presenting with AFP-negative HCC. This diagnostic gap underscores the imperative for novel, reliable biomarkers to facilitate early detection and improve patient outcomes. Thioredoxin (Trx), a key redox-regulating protein, has emerged as a compelling candidate. Overexpressed in response to oxidative stress—a hallmark of hepatocarcinogenesis—Trx is secreted into the bloodstream by malignant hepatocytes, implicating it directly in tumor progression, proliferation, and apoptosis evasion. This comprehensive review synthesizes the extant evidence elucidating the diagnostic and prognostic utility of serum Trx specifically within the challenging context of AFP-negative HCC. We critically appraise clinical studies demonstrating that serum Trx levels are significantly elevated in patients with AFP-negative HCC compared to those with liver cirrhosis, chronic hepatitis, and healthy controls. The review delves into the pathophysiological mechanisms linking Trx overexpression to hepatocarcinogenesis, including its interaction with key signaling pathways such as ASK1/p38 and NF-κB. Furthermore, we evaluate the performance of Trx as a standalone biomarker and its enhanced diagnostic accuracy when integrated into multi-marker panels alongside other emerging biomarkers like Dickkopf-1 (DKK1) and Glypican-3 (GPC3). The analysis also addresses the prognostic value of serum Trx, correlating elevated levels with tumor stage, metastasis, and survival. Despite promising data, we identify limitations in the current body of literature, including heterogeneity in assay methodologies and the need for large-scale, prospective validation studies. In conclusion, serum Thioredoxin represents a highly promising diagnostic and prognostic tool capable of addressing a critical unmet need in the management of AFP-negative HCC, potentially paving the way for more personalized and effective diagnostic strategies.

**Keywords:** *Thioredoxin, Alpha-Fetoprotein, Negative Hepatocellular Carcinoma*

10.48047/jocaaa.2024.33.06.127

# INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and a leading cause of cancer-related death worldwide.[1] Its incidence continues to rise, driven largely by the burden of chronic liver diseases such as hepatitis B and C virus infections, non-alcoholic fatty liver disease (NAFLD), and alcohol-related liver disease.[2] The prognosis of HCC is starkly stratified by the stage at diagnosis; while curative options like resection or transplantation are available for early-stage disease, advanced HCC carries a dismal five-year survival rate.[3] This underscores the paramount importance of effective surveillance and early diagnostic modalities.

For decades, serum alpha-fetoprotein (AFP) has been the cornerstone of HCC biomarker diagnosis. Integrated into major clinical practice guidelines, its measurement is widespread due to its accessibility and low cost.[4] However, the diagnostic performance of AFP is hampered by significant limitations. Its sensitivity for detecting early-stage HCC is suboptimal, ranging from 40% to 65%, and its specificity is compromised by elevations in various benign chronic liver diseases, including active hepatitis and cirrhosis.[5] Most critically, approximately 30-40% of HCC patients do not exhibit elevated AFP levels, a cohort classified as having AFP-negative HCC.[6] This substantial patient population remains diagnostically vulnerable, often facing delays in diagnosis and intervention.

Consequently, the quest for novel, complementary biomarkers is a pressing priority in hepatology and oncology research. The ideal biomarker would demonstrate high sensitivity and specificity for HCC, particularly in its early stages and within the AFP-negative subgroup. In this context, the molecular pathways of hepatocarcinogenesis offer a fertile ground for discovery. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, is a well-established driver of liver damage, fibrosis, and ultimately, carcinogenesis.[7] It promotes DNA damage, genomic instability, and activates pro-oncogenic signaling pathways.

Thioredoxin (Trx) is a fundamental, ubiquitous redox-active protein of 12-kDa that plays a central role in maintaining cellular redox homeostasis.[8] Beyond its intracellular antioxidant functions, Trx is secreted by cells in response to oxidative stress and inflammatory stimuli. Notably, Trx is overexpressed in a wide range of human cancers, including HCC, where it contributes to tumor cell growth, survival, and resistance to chemotherapy.[9] The detectable presence of Trx in the serum of cancer patients has positioned it as a potential circulating biomarker.

This comprehensive review aims to critically evaluate the existing body of evidence regarding the role of serum Thioredoxin as a diagnostic marker specifically for AFP-negative hepatocellular carcinoma. We will explore its pathophysiological rationale, summarize clinical studies assessing its diagnostic and prognostic accuracy, and discuss its potential integration into future multi-marker diagnostic algorithms to bridge the current gap in HCC management.

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## 2. Pathophysiological Rationale: Linking Oxidative Stress, Thioredoxin, and Hepatocarcinogenesis

The elevation of serum Thioredoxin in HCC is not an epiphenomenon but is deeply rooted in the molecular pathogenesis of the disease. The progression from chronic liver injury to cirrhosis and ultimately to HCC is fueled by a milieu of persistent oxidative stress, chronic inflammation, and compensatory regeneration. **[10]** Within this milieu, the Thioredoxin system emerges as a critical player.

a. **The Thioredoxin System: A Primer**

b. The Thioredoxin system is a major antioxidant system comprising Thioredoxin (Trx), Thioredoxin reductase (TrxR), and NADPH. **[11]** Its primary function is to regulate the intracellular redox state by reducing disulfide bonds in target proteins, thereby controlling their activity. Trx itself exists in both intracellular (Trx-1) and mitochondrial (Trx-2) forms, with the 12-kDa Trx-1 being the isoform detected in serum. **[8]** Under severe oxidative stress, mammalian cells actively secrete Trx-1, although the precise mechanism of secretion remains an area of active investigation. **[12]**

### 2.2. Oxidative Stress as a Driver of HCC

In chronic liver diseases, the sustained generation of Reactive Oxygen Species (ROS) from damaged hepatocytes, activated Kupffer cells, and infiltrating inflammatory cells leads to lipid peroxidation, protein modification, and DNA damage. **[7]** This creates a selective pressure for the clonal expansion of hepatocytes with mutations that confer survival and growth advantages. Key oncogenic pathways, such as the p53 tumor suppressor pathway, are particularly vulnerable to oxidative inactivation, facilitating uncontrolled proliferation. **[13]**

### 2.3. The Dual Role of Thioredoxin in Liver Cancer

In the context of HCC, Trx overexpression serves a dual, pro-tumorigenic purpose:

- **Cytoprotective Survival Signal:** By scavenging ROS, Trx protects malignant and pre-malignant hepatocytes from oxidative stress-induced apoptosis. This is mechanistically achieved through the reduction and inhibition of Apoptosis Signal-regulating Kinase 1 (ASK1). **[14]** In its oxidized state, Trx binds to ASK1, inhibiting its pro-apoptotic activity. Upon reduction, Trx dissociates from ASK1, allowing it to activate the p38 and JNK pathways leading to apoptosis. In HCC cells with high Trx levels, ASK1 is perpetually suppressed, conferring a potent survival advantage. **[15]**
- **Pro-Proliferative Signal:** Trx directly influences transcription factors critical for cell growth and survival. It enhances the DNA-binding activity of NF-κB, a master regulator of inflammation and cell proliferation, and Ref-1-dependent activation of AP-

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1. **[16]** Furthermore, Trx can promote growth factor expression and stimulate angiogenesis, thereby supporting tumor expansion and metastasis. **[17]**

The overexpression of Trx in HCC tissue, confirmed by numerous immunohistochemical studies, directly translates into elevated serum levels. **[18]** This secretion is believed to be a consequence of both active release from stressed and malignant hepatocytes and passive release from necrotic cells within the tumor microenvironment. Therefore, serum Trx level serves as a quantifiable reflection of the intense oxidative and proliferative drive within the hepatic tumor, providing a strong pathophysiological basis for its use as a diagnostic biomarker, especially in AFP-negative tumors where alternative oncogenic drivers may be more dependent on redox-sensitive pathways.

## 3. Diagnostic Performance of Serum Thioredoxin in AFP-Negative HCC

The translation of the robust pathophysiological rationale for Thioredoxin into clinical utility is supported by a growing body of evidence demonstrating its discriminative power, particularly in the diagnostically challenging cohort of AFP-negative HCC patients. This section synthesizes findings from key clinical studies that have specifically investigated the diagnostic accuracy of serum Trx.

### 3.1. Early Pioneering Studies and Foundational Evidence

Initial investigations established the fundamental link between serum Trx levels and HCC. A landmark case-control study by **Yamamoto et al.** demonstrated that serum Trx levels were significantly higher in HCC patients compared to those with chronic hepatitis, liver cirrhosis, and healthy controls. Crucially, this study also included a subgroup analysis revealing elevated Trx in a proportion of patients with small, resectable tumors, hinting at its potential for early detection. [19]

Subsequent research honed in on the AFP-negative population. A pivotal study by **Zhou et al.** systematically evaluated serum Trx in a cohort comprising healthy controls, patients with chronic hepatitis B, liver cirrhosis, and HCC. Their findings were striking: while Trx levels were elevated in chronic liver disease, they were significantly higher in the HCC group. Most importantly, in the subgroup of patients with AFP-negative HCC (AFP < 20 ng/mL), serum Trx maintained a high diagnostic value, with an Area Under the Receiver Operating Characteristic Curve (AUC) of 0.85 for distinguishing HCC from cirrhosis. [20] This provided one of the first direct evidence that Trx could effectively identify HCC cases missed by standard AFP testing.

### 3.2. Comparative and Combinatorial Diagnostic Accuracy

Later studies have further solidified this role, often comparing Trx to other emerging biomarkers. Research has consistently reported that serum Trx outperforms AFP in terms of sensitivity for detecting HCC overall, and this advantage is most pronounced in the AFP-negative subgroup. [21] For instance,

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a study by **Liu et al.** reported that the sensitivity of Trx for diagnosing AFP-negative HCC was approximately 75%, significantly surpassing the by-definition 0% sensitivity of AFP in this group. [22] The diagnostic power of Trx is further enhanced when used in combination with other biomarkers. The combination of Trx and AFP has been shown to yield a higher AUC and improved sensitivity than either marker alone, effectively capturing a broader spectrum of HCC patients. [23] Even more compelling for the context of this review is the performance of Trx within multi-marker panels that exclude AFP. Panels combining Trx with other novel biomarkers such as Dickkopf-1 (DKK1) or Glypican-3 (GPC3) have demonstrated superior diagnostic efficacy for AFP-negative HCC, with AUCs often exceeding 0.90. [24, 25] This suggests that Trx is a cornerstone biomarker in a new, AFP-independent diagnostic algorithm.

### 3.3. Distinguishing HCC from Benign Liver Disease

A critical challenge for any HCC biomarker is to differentiate malignancy from benign, yet active, chronic liver disease. Studies indicate that while serum Trx is modestly elevated in cirrhosis and severe hepatitis, the levels in HCC are significantly higher. [20, 26] The optimal diagnostic cut-off value for Trx varies between studies (often reported between 30-50 ng/mL depending on the assay used), but consistently, the AUC for distinguishing HCC from cirrhosis falls in the range of 0.80-0.90, indicating good to excellent diagnostic accuracy. [27]

In summary, the collective evidence strongly positions serum Thioredoxin as a sensitive and reliable serological marker capable of identifying AFP-negative hepatocellular carcinoma with a high degree of accuracy, both as a standalone test and as a pivotal component of a next-generation biomarker panel.

## 4. Prognostic and Therapeutic Implications of Serum Thioredoxin

The clinical utility of a biomarker extends beyond diagnosis into prognostication and guiding therapy. Emerging data indicate that serum Thioredoxin levels hold significant value not only in identifying AFP-negative HCC but also in predicting tumor behavior, patient outcomes, and potential therapeutic vulnerabilities.

### 4.1. Correlation with Tumor Burden and Disease Progression

Multiple clinical studies have demonstrated a positive correlation between circulating Trx levels and established indicators of advanced disease. Elevated serum Trx concentrations have been significantly associated with larger tumor size, multi-nodularity, and the presence of vascular invasion and extrahepatic metastases. **[28]** A longitudinal study by **Wang et al.** found that rising serum Trx levels over time were predictive of disease progression and the development of metastatic lesions in a cohort that included AFP-negative patients. **[29]** This suggests that serial monitoring of Trx could serve as a dynamic marker for tracking tumor aggressiveness and response to treatment.

### 4.2. Serum Thioredoxin as a Prognostic Indicator for Survival

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The association between high pre-treatment serum Trx levels and unfavorable survival outcomes is a consistent finding across several investigations. Patients with elevated Trx at diagnosis, regardless of their AFP status, have been shown to have significantly shorter overall survival and higher recurrence rates following curative-intent treatments such as surgical resection or radiofrequency ablation. **[30, 31]** For example, a multivariate Cox regression analysis by **Li et al.** identified a serum Trx level above 40 ng/mL as an independent prognostic factor for poor overall survival, after adjusting for Child-Pugh stage, tumor size, and AFP level. **[32]** This positions Trx as a powerful, independent prognostic tool that can aid in risk stratification and patient counseling.

### 4.3. Implications for Therapy and the Thioredoxin System as a Therapeutic Target

The mechanistic role of Trx in promoting cell survival and treatment resistance directly informs its prognostic value. High Trx expression confers resistance to a range of chemotherapeutic agents and to radiation therapy by mitigating oxidative stress-induced apoptosis, which is a key mechanism of action for many cancer treatments. **[33]**

This very role, however, reveals a therapeutic opportunity. The Thioredoxin system has become an attractive target for novel anticancer drugs. Inhibitors of Thioredoxin reductase, such as Auranofin (a gold-containing compound), and direct Trx inhibitors are under active investigation in preclinical and early clinical trials. **[34, 35]** The measurement of serum Trx could, therefore, evolve into a predictive biomarker for identifying patients most likely to benefit from therapies targeting the redox system. A tumor reliant on high Trx activity for survival may be particularly vulnerable to its inhibition, a concept known as "redox synthetic lethality." **[36]**

In conclusion, the prognostic utility of serum Thioredoxin enriches its diagnostic value, providing a window into the biological aggressiveness of HCC. Its integration into clinical practice could help identify AFP-negative patients with a high risk of progression, inform surveillance intensity, and potentially guide the selection of novel targeted therapies in the future.

## 5. Comparative Analysis with Other Novel Biomarkers for AFP-Negative HCC

The diagnostic challenge of AFP-negative HCC has spurred the investigation of numerous novel biomarkers. Placing Serum Thioredoxin within this broader context is essential to evaluate its relative standing and potential for integration into a multi-marker diagnostic strategy.

### 5.1. Established and Emerging Serological Biomarkers

Several other biomarkers have shown promise in diagnosing AFP-negative HCC:

- **Lens culinaris-agglut**

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**inin-reacti ve fracti on of AFP (AFP-L3):** T his glycof orm of AFP is often consid ered more specifi c for HCC than total AFP. Howe ver, its sensiti vity, particu larly for early-stage and AFP-negati

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ve HCC, remains a limitation, as it requires a baseline level of AFP to be measurable. **[37]**

- **Des-gamma-carboxy prothrombin (DCP/ PIVKA-II):** DCP has gained widespread clinica

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l acceptance and is incorporated into some guidelines. It demonstrates good specificity but can be elevated in vitamin K deficiency and has variable sensitivity for early-stage HCC. Its

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perfor mance is often compl ement ary to, but not necess arily superi or to, Trx in the AFP-negati ve contex t. **[38]**

- **Glypi can-3 (GPC 3):** An oncofe tal protei n overex presse d in HCC tissue. Serum GPC3

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and its autoantibodies show high specificity but modest and variable sensitivity (often 50-70%), limiting its use as a standalone test. **[39]** The pathophysiological basis of GPC3 (a cell-surface

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proteoglycan) is distinct from that of Trx (a secreted redox protein), suggesting non-overlapping diagnostic information.

- **Dickkopf-1 (DKK1):** A secretory protein involved in the Wnt signali

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ng pathway. Serum DKK1 has demonstrated good diagnostic performance for HCC, including in AFP-negative cases. **[40]** Like Trx, it represents a different oncogenic pathway, providing an

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### 5.2. The Superiority of a Multi-Marker Panel

The consensus emerging from the literature is that no single biomarker is likely to replicate the ideal combination of high sensitivity and high specificity for AFP-negative HCC. The future of serological diagnosis lies in rationally constructed multi-marker panels. **[41]**

In this paradigm, Serum Thioredoxin holds a strong position due to its unique basis in the oxidative stress pathway. When combined with biomarkers reflecting other hallmarks of cancer—such as **DKK1 (dysregulated Wnt signaling)**, **GPC3 (altered differentiation)**, and **DCP (impaired vitamin K metabolism)**—the resulting panel can capture the pathophysiological heterogeneity of HCC. **[42]**

Studies that have directly compared panels are illustrative. For instance, a panel combining **Trx, DKK1, and GPC3** was shown to significantly outperform any single marker for diagnosing early-stage HCC, with an AUC exceeding 0.95 and maintaining high sensitivity in AFP-negative patients. **[25, 43]** This synergistic effect confirms that these biomarkers are not redundant but are detecting complementary aspects of hepatocarcinogenesis.

Therefore, while Serum Thioredoxin is a powerful standalone biomarker, its greatest clinical value may be realized as a core component of a multi-analyte panel, effectively creating a "serological fingerprint" for AFP-negative hepatocellular carcinoma that is far more accurate than the sum of its parts.

## 6. Limitations, Challenges, and Future Directions

Despite the compelling evidence supporting the diagnostic and prognostic utility of serum Thioredoxin in AFP-negative HCC, several significant challenges must be acknowledged and addressed before its widespread clinical adoption can be realized.

### 6.1. Standardization of Assay Methodologies

A primary obstacle is the current lack of a standardized, universally accepted assay for quantifying serum Trx. Studies to date have employed various immunoassay techniques, including enzyme-linked immunosorbent assays (ELISAs) from different commercial manufacturers and in-house developed assays. **[44]** This heterogeneity leads to substantial inter-study variation in reported absolute Trx concentrations and proposed diagnostic cut-off values. The establishment of a reference measurement procedure and international standards is a critical prerequisite for comparing results across institutions and validating findings in large, multi-center trials. **[45]**

### 6.2. Specificity in the Context of Systemic Conditions

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While serum Trx effectively distinguishes HCC from cirrhosis in many studies, its elevation is not entirely specific to liver cancer. Increased levels have been documented in a range of other pathological states characterized by systemic oxidative stress and inflammation, including rheumatoid arthritis, HIV infection, and other solid malignancies. **[46, 47]** This underscores the importance of interpreting serum Trx levels within the specific clinical context of a patient with chronic liver disease, and it reinforces the necessity of using it as part of a panel rather than a standalone test.

### 6.3. Need for Robust Prospective Validation

The majority of existing evidence comes from case-control studies, which are inherently susceptible to spectrum bias. There is a pressing need for large-scale, prospective, longitudinal cohort studies that enroll patients with cirrhosis—the highest-risk population—and follow them over time with serial Trx measurements. **[48]** Such studies are essential to definitively establish the biomarker's performance for *early detection* of HCC, its lead time before radiographic diagnosis, and its cost-effectiveness within a structured surveillance program.

### 6.4. Future Research Directions

Future investigations should focus on several key areas:

- **Prospective Validation:** Initiating the large-scale cohort studies as described above.
- **Panel Refinement:** Conducting sophisticated statistical analyses (e.g., machine learning models) to determine the optimal combination of Trx with other biomarkers (e.g., DKK1, GPC3, microRNAs) and clinical parameters to maximize diagnostic yield.
- **Therapeutic Monitoring:** Exploring the role of serial Trx measurement in monitoring response to locoregional and systemic therapies, and its potential as a predictive biomarker for Trx system-targeted therapies.
- **Mechanistic Links:** Further elucidating the precise cellular mechanisms governing Trx secretion from pre-malignant and malignant hepatocytes.

In conclusion, while serum Thioredoxin is a highly promising biomarker, its journey from research to routine clinical practice hinges on overcoming these standardization and validation hurdles. The path forward is clear and necessitates a concerted, collaborative effort from the scientific community.

## 7. Conclusion

The high prevalence of AFP-negative hepatocellular carcinoma represents a critical diagnostic dilemma in clinical hepatology, directly contributing to delayed diagnosis and poor patient outcomes. This comprehensive review has synthesized the substantial and growing body of evidence establishing serum Thioredoxin as a biomarker of high diagnostic and prognostic value specifically for this

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challenging patient subgroup. The pathophysiological foundation is robust: Trx overexpression is a direct molecular consequence of the oxidative stress that drives hepatocarcinogenesis, and its secretion into the serum provides a measurable reflection of this key tumorigenic process.

Clinical data consistently demonstrate that serum Trx levels are significantly elevated in patients with AFP-negative HCC, enabling effective differentiation from benign chronic liver diseases with good to excellent accuracy. Its utility is further enhanced when integrated into multi-marker panels, where it acts synergistically with biomarkers from other pathways, such as DKK1 and GPC3, to create a powerful serological profile for AFP-negative tumors. Beyond diagnosis, serum Trx level serves as an important prognostic indicator, correlating with tumor aggressiveness, metastatic potential, and overall survival, thereby aiding in risk stratification.

However, the translation of this promising biomarker into routine clinical practice requires overcoming clear challenges. The lack of a standardized assay and the need for validation in large, prospective surveillance cohorts are the most pressing issues. Future research must focus on addressing these limitations and refining the role of Trx within evolving diagnostic algorithms. In summary, serum Thioredoxin has unequivocally emerged as a pivotal tool, holding the potential to bridge a long-standing diagnostic gap and pave the way for more personalized and effective management strategies for patients with AFP-negative hepatocellular carcinoma.

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